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Cyclodextrin derivatives containing covalently bound volatile substances and studies of their release

Cyklodextrinové deriváty obsahující kovalentně vázané těkavé látky a studium jejich uvolňování

Bachelor thesis

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Prague, 2018

Declaration

I declare that I have written my bachelor thesis by myself and that all the sources are listed in the bibliography. Neither this work, nor its significant part was used to obtain other academic title. I agree that this work may be lent and published.

Prague, 22.05.2018 Attila Palágyi

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Abstract

The main aim of the work was to prepare a series of β-cyclodextrin derivatives containing covalently bound volatile compounds via an imine bond. The used volatile compounds were cinnamaldehyde, cyclamen aldehyde, lilal, benzaldehyde, anisaldehyde, vanillin, hexanal, heptanal, citral and 5-methylfurfural. Subsequently, the rate of the release of the active compound, as a function of the environment, was studied by ^{1}H NMR spectroscopy and static headspace-gas chromatography.

Key words: cyclodextrin, aldehyde, imine, kinetics, controlled release

Abstrakt

Hlavním cílem práce bylo připravit řadu β-cyklodextrinových derivátů obsahujících kovalentně vázané těkavé sloučeniny přes iminovou vazbu. Jako těkavé látky byly použity cinnamaldehyd, cyklamenaldehyd, lilal, benzaldehyd, anisaldehyd, vanilin, hexanal, heptanal, citral a 5-methylfurfural. Následně byla zkoumána rychlost uvolňování účinné látky jako funkce prostředí pomocí ^{1}H NMR spektroskopie a SHGC (static headspace-gas chromatography).

Klíčové slova: cyclodextrin, aldehyd, imin, kinetika, řízené uvolňování

Contents

List of abbreviations

CD – cyclodextrin

COSY – correlated spectroscopy

DMF – dimethylformamide

DMSO – dimethylsulfoxide

EM – eluent mixture

eq. – equivalent

ESI – electrospray ionization

G – guest

GC – gas chromatography

HMBC – heteronuclear multiple bond coherence

HSQC – heteronuclear single quantum coherence

IC – inclusion complex

K – partition (distribution) coefficient or equilibrium constant

K_f – formation constant

Me – methyl

MHE – multiple headspace extraction

M_r – standard molecular weight

NMR – nuclear magnetic resonance

SHGC – static headspace-gas chromatography

TLC – thin layer chromatography

VOC – volatile organic compound

1 Introduction

Fragrance and flavor industry is one of the most intensively developing sectors of chemical industry. Encapsulation techniques are widely used in both, food and cosmetic industries, not just to control the delivery of the encapsulated guest molecules but also to protect those agents from environmental degradation. Cyclodextrins (CDs) serve as one of the simplest encapsulating systems. CDs are cyclic oligosaccharides composed of 1-4 linked α-*D*-glucopyranose units (6, 7, and 8 for α-, β-, and γ-CD). CDs are well known for their hydrophilic outer surface and hydrophobic cavity. This cavity can encapsulate another lipophilic guest molecule and therfore form an inclusion complex[1,2].

A staggering number of inclusion complexes of CDs with various organic molecules have been described so far [3,4]. It was proved that the complexation of volatile organic compounds, such as aldehydes, into the CD's cavity reduces the volatility and increases the solubility and bioavailability of these compounds. The release of the bound molecule from the CD's cavity takes from minutes to hours, depending on the environmental conditions as well as on the structure of the molecule. The prolongation of the release time of the complexed compounds would make significant progress in fragrance delivery as well as in odor and flavor control. It is considered an important move to improve the stabilization, quality, efficiency and the persistence of repellents, disinfectants, perfumes, laundry detergents and flavoring agents.

In this bachelor thesis, we describe the synthesis of ten Schiff bases of 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin and aldehydes. The imine bond was chosen because it is relatively stable; on the other hand, it can be readily hydrolyzed, forming the amino-β-cyclodextrin and releasing the aldehyde. The kinetics of the aldehyde release was studied by NMR techniques in buffers with different pH values. The aldehyde release itself from the buffers and by humidity was studied by static headspace-gas chromatography.

2 The aims of the work

The main goal of this bachelor thesis is to synthesize a series of novel β-cyclodextrin derivatives containing covalently bound aldehydes via an imine bond as pro-fragrances. In the next phase, the prepared derivatives will be subjected to hydrolysis, and the release of the fragrances will be studied by NMR and static headspace-gas chromatography (SHGC).

The synthetic process can be summarized into following steps:

- Monotosylation of β-CD in position 6
- Substitution of the *p*-toluenesulfonate group with an azide group
- Reduction of the azide to amine
- Preparation of Schiff bases of 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin and aldehydes (cinnamaldehyde, cyclamen aldehyde, lilal, benzaldehyde, anisaldehyde, vanillin, hexanal, heptanal, citral and 5-methylfurfural)

The study of the release of the aldehydes from the prepared compounds can be summarized into following steps:

- Kinetic studies of the hydrolysis in buffers with different pH by ^{1}H NMR spectroscopy
- Study of the fragrance release in buffers with different pH by SHGC
- Study of the fragrance release at different humidity by SHGC

3 Theory

3.1 Cyclodextrins

The first mention of substances later called cyclodextrins is from 1981. Antoine Villiers, a French analytical chemist, isolated a white crystalline which he named "cellulosine" because of its similarity [5]. "Cellulosine" was obtained from potato starch by enzymatic degradation with butyric ferment *Bacillus amylobacter* (*Clostridium Butyricum*). This bacterial culture was probably a coculture with another bacillus (*Bacillus macerans*[6]), which caused the formation of Villier's byproduct. Villiers also noticed that the "cellulosine" is a mixture of two different compounds – it was probably α-cyclodextrin and β-cyclodextrin[1].

In 1903 Franz Schardinger, an Austrian chemist and bacteriologist, has obtained the same white crystalline products as Villiers while studying the degradation of starch by microorganisms. Later he proved that the formation of the "cellulosine" is caused by *Bacillus macerans*. This was the beginning of the cyclodextrin chemistry. In the 1930s a procedure of isolating pure cyclodextrin was described, and its cyclic structure was assumed. Later studies proved the cyclic structure of cyclodextrins, and also some of their physical and chemical properties were described[1].

At the end of the 1960s, the cyclodextrins were known as important substances not just from the industrial aspect, but also for their potential use in pharmaceutical and food industry, mostly because of their nontoxicity[1].

Nowadays, native cyclodextrins are produced by enzymatic degradation of starch and its derivatives, which are inexpensive starting material. The enzyme involved in the degradation is called cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19), which is an enzyme produced by bacteria such as *Bacillus macerans*, *Bacillus circulans*, *Alkalophylic bacillus*, *Klebsiella oxytoca* and more. The crude product is mainly a mixture of α- β- and γ-cyclodextrin[7]. β-Cyclodextrin is the easiest to separate due to its low solubility in water, which makes it economically and practically the most available with the highest utilization and marketing potential[8].

3.1.1 Structure and properties

Cyclodextrins (CDs) are cyclic oligosaccharides composed of *D*-glucopyranose units in $^4C_1$ conformation mutually interconnected by α-(1→4) glycosidic bonds. This is the reason why they are also classified as nonreducing sacharides and are also refered to as cyclomaltoses, cycloamyloses, cycloglucopyranoses or Schardinger dextrins. CDs naturally form white crystalline powder, amongst which the most common are α-CD, β-CD and γ-CD with 6, 7 and 8 α-*D*-glucopyranose units respectively[8] (Figure 1). However, CDs with lower (pre-α-CD with 5 glucose units)[9,10] and higher, δ-, ε-, ζ-, η-, and θ-CDs with 9, 10, 11, 12 and 13 glucose units respectively were also reported[11].

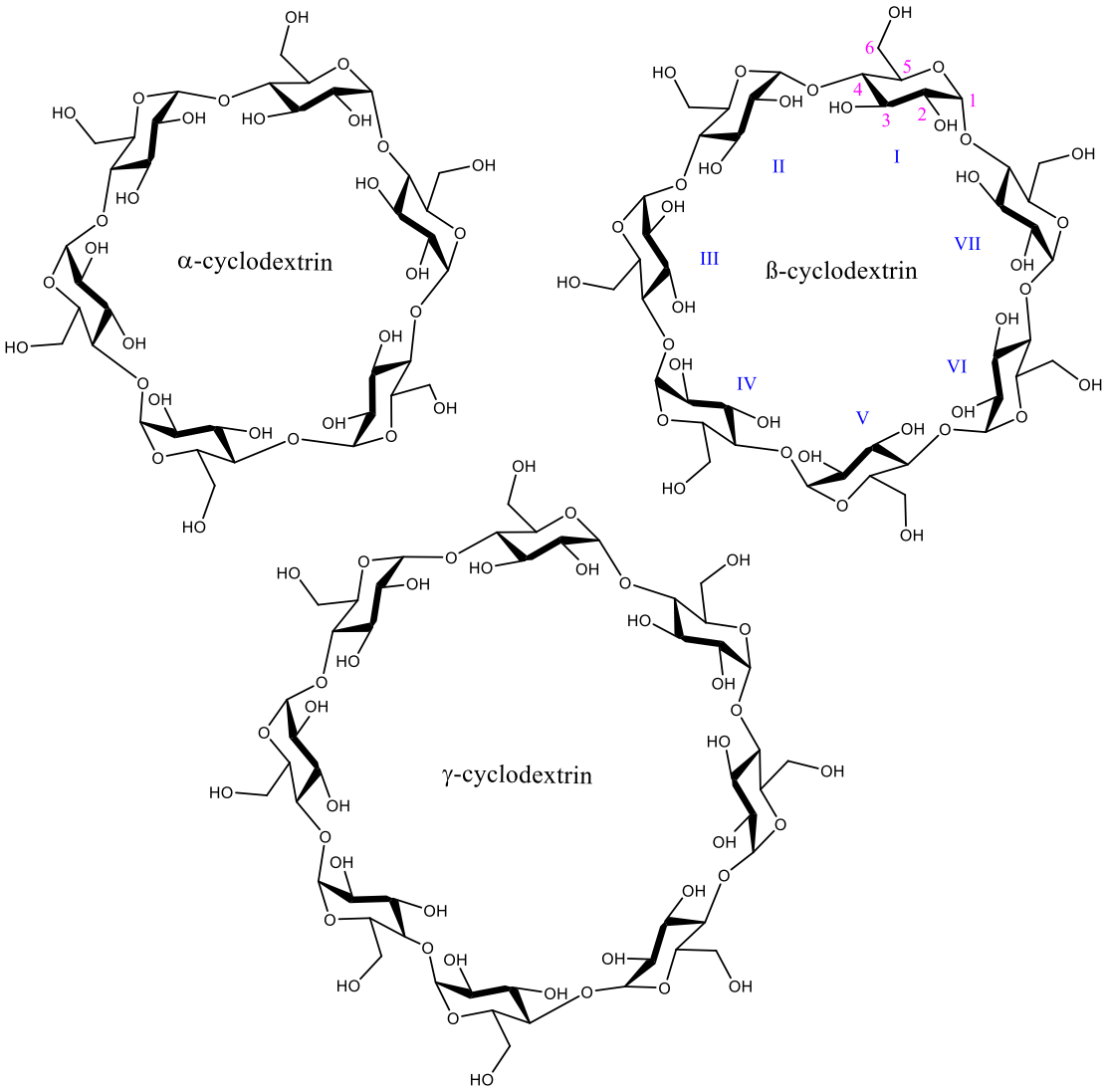


Figure 1: The molecular structures of α-, β- and γ-cyclodextrins with example of glucose atom numbering (purple Arabic numbers) and glucose unit numbering (blue Greek numbers).

Due to the $^4C_1$ conformation of the α-D-glucopyranose units, the CDs have a shape of a hollow truncated cone (conical cylinder) with a hydrophobic cavity and hydrophilic outer surface. Secondary hydroxyl groups on C2 and C3 are located on the wider – secondary rim of the cavity, while primary hydroxyl groups on C6 are located on the other side of the CD molecule – on the primary rim (Figure 2)

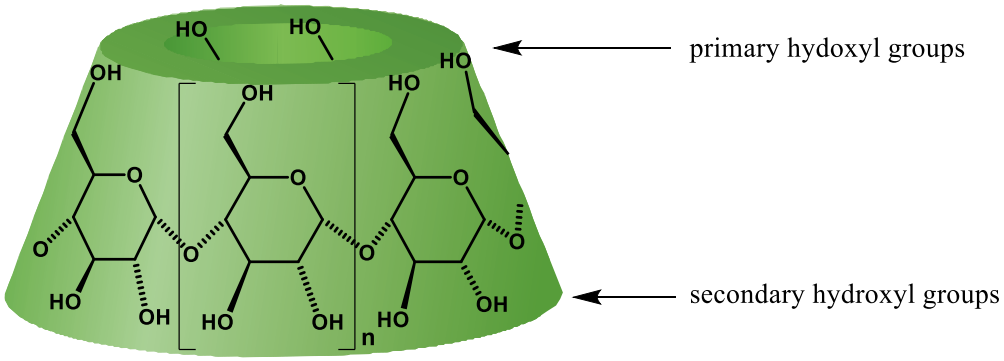


Figure 2: The molecular shape of α-CD (n = 1), β-CD (n = 2), γ-CD (n = 3)

On account of outer hydrophilic character, CDs are well soluble in polar solvents as water, DMSO, DMF, etc. Hydroxyl groups on C3 can form hydrogen bonds with the hydroxyl groups on C2. In β-CD, those hydrogen bonds form a whole stable belt causing the rigidity of its molecule which results in the low solubility of β-CD in water. In the case of α-CD with six α-*D*-glucopyranose units, the hydrogen bond belt is less compact, so it has more tendencies to interact with water molecules. On the other hand, the hydrogen bond belt of γ-CD with its eight α-D-glucopyranose units is less stable due to its greater macrocycle and flexibility emerging in its highest aqueous solubility of the three CDs.

Native CDs are relatively stable in aqueous solutions. At pH 12.1 and higher their hydroxyl groups start to deprotonate and in acidic media with the pH value lower than 3 at elevated temperature above 60 °C the CDs start to hydrolyze. Some of the tabbed physical and chemical properties of native CDs are summarized in Table 1[2].

Table 1: Selected properties of native cyclodextrins[2]

	α-CD	β-CD	γ-CD
Number of glucose units	5	6	7
Molecular weight [g·mol^{-1}]	973	1135	1297
Cavity diameter at secondary rim [nm]	0.47-0.53	0.60-0.65	0.75-0.83
CD diameter at secondary rim [nm]	1.46 ± 0.04	1.54 ± 0.04	1.75 ± 0.04
Height of torus [nm]	0.79 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
Cavity volume [nm^3]	0.174	0.262	0,427
Cavity volume of 1 mol CD [ml]	104	157	256
Number of water molecules in cavity	6	11	17
Crystal water [wt %]	10.2	13.2-14.5	8.3-17.7
Aqueous solubility [mg·ml^{-1}] at 25 °C	145	18.5	232
pK_A at 25 °C	12.33	12.20	12.08
Optical rotation $[\alpha]^{25}_D$	150 ± 0.5	162.5 ± 0.5	177.4 ± 0.5

3.1.2 Inclusion complexes

Apparently amongst the most important and the most useful features of the CDs is the ability to form inclusion complexes (ICs) with a wide range of molecules in solid, liquid or gaseous phase. The above mentioned supramolecular inclusion phenomenon is often referred to as host-guest complexation. To form a stable inclusion complex, the guest molecules have to be relatively nonpolar and have adequate size and shape to fit into the CD's cavity [12].

In aqueous solutions, CDs can host a vast amount of organic compounds of lipophilic character such as linear or branched hydrocarbons, alcohols, aldehydes, ketones, organic acids, aromatics, amines, etc. Besides the above-mentioned complexes of CDs complexes with noble gases, ionic and neutral inorganic compounds and complexes in gaseous phase have also been described[4,13]. In general, α-CDs form the most stable complexes with molecules containing aliphatic chain (e.g., heptanal, octanol), β-CDs with compounds having aromatic functional groups (e.g., cinnamaldehyde, toluene) and γ-CDs prefer larger molecules (e.g., pyrene, fullerene). Noncovalent bonds are broken or formed during the complexation process[14].

Cyclodextrins form inclusion complexes with guests in different ratios. The most common host:guest ratios are 1:1, 1:2 and 2:1, but other ratios have been also described [15,16].

The complexation of the guest molecule by the CD has a significant impact on its physical and chemical properties. In the case of an aqueous solution of CD and a poorly soluble guest, the following effects can be observed.

- The concentration of solubilized guest molecules increases due to the formation of ICs with CD but on the other hand, the solubility of the CD can decrease[17].
- The reactivity of the guest generally decreases as a result of the protection from the surrounding environment. In some cases the CD can figure as a catalyst, therefore the reactivity of the guest can be increased[18].
- The guest's volatility decreases[19].
- Changes in spectral data. For example changes in chemical shifts of anizotropically shielded cores in NMR spectra[20,21].

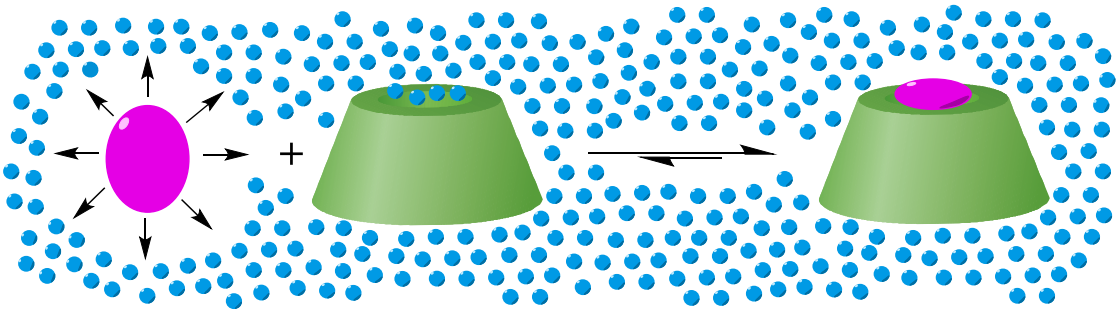


Figure 3: Complexation of a hydrophobic molecule by cyclodextrin

The main driving force of formation of ICs is in the high energy water molecules in the CD's cavity, which have high enthalpy and their presence in the cavity is energetically not favored. In the presence of a suitable guest molecule, the unfavored water molecules present in the cavity are replaced by the lipophilic guest forming a new, more stable lower energy state[22,23]. Alongside in formation of ICs other forces also play their roles such as entropy[23], hydrogen bonding, van der Waal's forces, electrostatic interactions, hydrophobic interactions and changes in solvent-surface tensions, and the release of a ring strain in CD molecule[24,25].

The formation of ICs is a dynamic equilibrium process. Most often the resulting ICs are in 1:1 ratio. In that case the following thermodynamic equilibrium can be assumed, where [*G*] and [CD/*G*] symbolize the concentration of the guest and the IC:

$$[\mathrm{CD}] + [\mathrm{G}] \rightleftarrows [\mathrm{CD/G}] \tag{3.1}$$

The value of the equilibrium shown above can be expressed with the formation constant K_f (also often called as stability, binding or complexation constant), w[26].

$$K_f = \frac{[\mathrm{CD/G}]}{[\mathrm{G}][\mathrm{CD}]} \tag{3.2}$$

The values of formation constants range from 10^2 to 10^5 M^{-1}, which was mentioned by Connors[26]. There are a lot of ways to determine the experimental values of K_f, such as potentiometry[27], kinetic[28] and spectroscopic methods[29] or static headspace-gas chromatography for volatile organic compounds[30].

3.2 Substituted cyclodextrin derivatives

Cyclodextrins are used not just in their native forms but also as modified. By modifying the native CDs we try to improve their properties and expand the fields of their use. In the most cases, the modifications of the native CDs take place on the hydroxyl groups, from which α-CD contains eighteen, β-CD twenty one and γ-CD twenty four; therefore the numbers of potential derivatives are high. CD derivatives can be persubstituted, mono-, di-, and more substituted, but also randomly substituted[25].

The preparation of substituted CDs is not as easy as it seems to be. Because of the presence of high numbers of almost equally reactive hydroxyl groups, a lot of regioisomers can be prepared. There are also other factors which threaten the successful outcome of the synthesis. For instance, the facts that the CD's cavity can unexpectedly complex the reactants and inhibit or stop the reaction, the limited solubility of the CDs and their derivatives and frequently the difficulty of separation of the formed regioisomers and the purification of the product from the reactants and the solvent which are often complexed into the cavity [31].

There are three main ways to prepare CD derivatives[32]. The first one is direct persubstitution which is fast and easy to perform. As an example there is the per-iodo-β-cyclodextrin, which is, due to its high insolubility, easy to purify with simple

extraction.[33] In the case of the second main way, we have to use protective groups, to prevent unwanted reactions. For example to prepare 2,3-methylated-β-cyclodextrin we have to protect the hydroxyl groups on the C6 by silylation and after the hydroxyl groups on the C2 and C3 are methylated, we remove the protective groups. The third way includes every other way of modifying the CDs. The separation of the products is often difficult, and chromatography or other sophisticated methods are needed to be used. For instance, in the case of preparation of 6^{I}-*O*-*p*-toluenesulfonyl-γ-cyclodextrin we obtain a mixture of the unmodified CD, mono-, di-, tri- and more toluenesulfonylated (tosylated) CDs, which need to be separated on a reverse chromatographic column[34].

3.2.1 Monosubstituted cyclodextrin derivatives

When planning a monosubstitution on CDs, we have to consider two main facts: the nucleophilicity of the hydroxyl groups and the ability of the CD to complex the reagents. Three types of hydroxyl groups are present in the CD's molecule, which differ in reactivity. The hydroxyl groups on the C6 carbon are the most basic (and mostly the most nucleophilic), on the C2 carbon the most acidic and on the C3 carbon sterically the least available[32].

Also, a huge impact on the outcome of the reaction has the choice of the solvent, especially when it comes to the stability and the orientation of the reagent in the formed complex with the CD. If the complex is stable, the final result will be most affected by how the reagent is oriented in the cavity of the CD. On the other hand, if the complex is not stable, the reaction will be regulated by the nucleophilicity of the hydroxyl groups. Because of this phenomenon, the yields of the monosubstitution reactions are very low and are ranging up maximally to a few dozens of percent[32].

3.2.2 The use of monosubstituted cyclodextrins

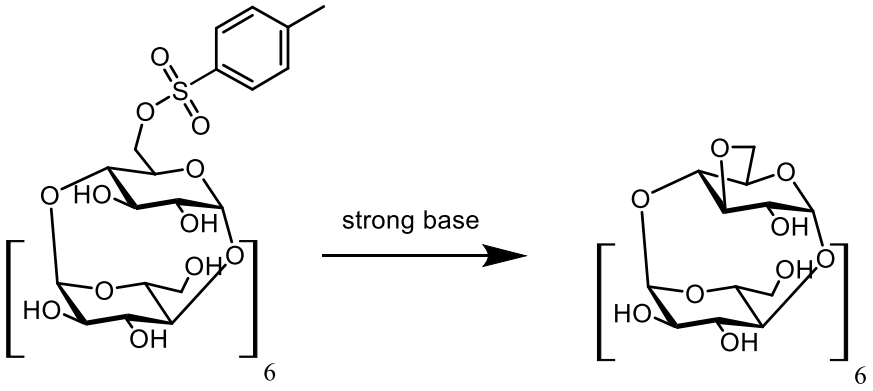
Monosubstituted CDs have a wide range of applications. In analytical chemistry, especially in capillary zone electrophoresis[35,35–37] and liquid chromatography[38,39], where they are used to separate structurally related substances or even to separate enantiomers.

Monoderivatives of CDs are also used in other human activities. For example, after binding of monosubstituted CDs to porous silicon, they can be used as photoluminescent sensors[40]. They can be used to synthesize a titanocene derivative which has antitumor effects or in other synthesis and also after binding to gold nanoparticles for subsequent extraction of [60]fullerene[41].

3.2.3 6^{I}-*O*-*p*-Toluenesulfonyl-cyclodextrin

The most frequently used monosubstitution reaction on the CDs is the substitution of one hydroxyl group on the C6 with an easily removable sulfonyl group. 6^{I}-*O*-Sulfonates, such as 6^{I}-*O*-*p*-toluenesulfonyl-cyclodextrins[42] are good precursors for the preparation of 6^{I}-deoxy-cyclodextrin derivatives by nucleophilic substitution of the sulfonyl group such as 6^{I}-azido-6^{I}-deoxy-β-cyclodextrin[43] and 6^{I}-amino-6^{I}-deoxy-

β-cyclodextrin[44]. It is not appropriate to use strong bases, because the chances of the formation of unwanted products, like 3,6-anhydro- cyclodextrin, can grow (Scheme 1)[45–47].



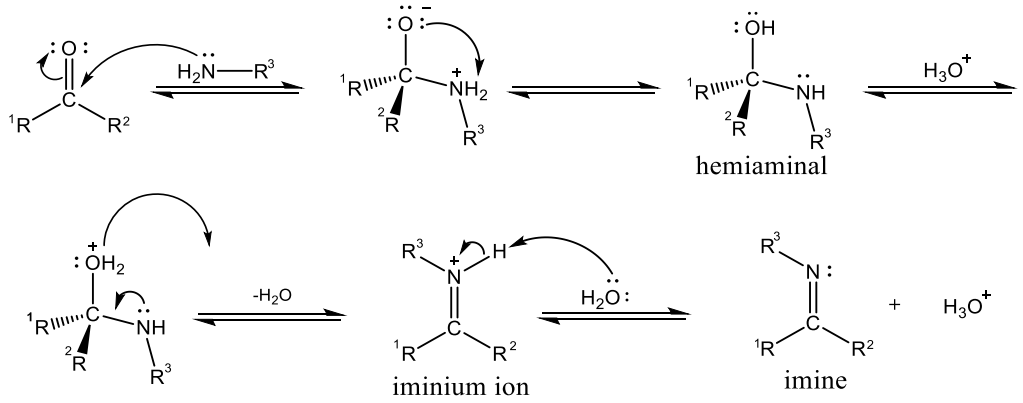
Scheme 1: Formation of 3,6-anhydro-β-cyclodextrin

3.3 Schiff bases

Imine is a functional group containing a carbon–nitrogen double bond. In general, the nitrogen atom can be attached to hydrogen (H) or an organic group (R). If this group is not a hydrogen atom, then the compound can be referred to as a Schiff base. A Schiff base, named after Hugo Schiff, is a compound with the general structure $R_2C{=}NR'$ ($R' \neq H$) and can be either a secondary ketimine or secondary aldimine, depending on its structure.

Formation of imines

Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound in a reversible, acid-catalyzed process. It begins with nucleophilic addition of the primary amine to the carbonyl group, followed by transfer of a proton from nitrogen to oxygen to yield a neutral amino alcohol, hemiaminal or carbinolamine. Protonation of the carbinolamine oxygen by an acid catalyst then converts the –OH group into a better leaving group H_2O^+– and iminium ion is produced by the elimination of a water molecule with E1 elimination mechanism. Loss of a proton from nitrogen gives the final product and regenerates the acid catalyst[48–51]. The mechanism is explained as shown in the Scheme 2.

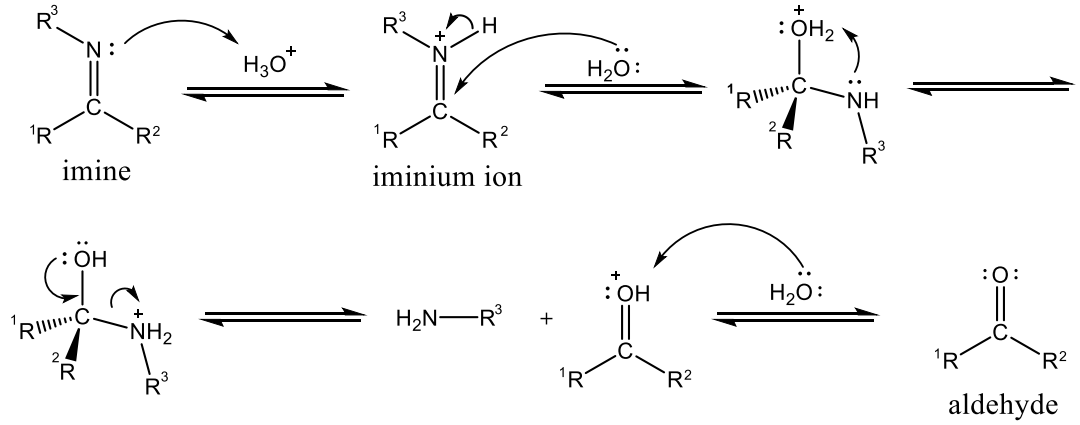


Scheme 2: The mechanism of imine formation

Imine formation is slow at both high pH and low pH but reach a maximum rate at a weakly acidic pH around 4 to 5[48]. We can explain the observed pH dependence of imine formation by looking at the individual steps in the mechanism. As indicated in Scheme 2, an acid catalyst is required in step 3 to protonate the intermediate carbinolamine, thereby converting the –OH into a better leaving group. Thus, the reaction will be slow if not enough acid is present (that is, at high pH). On the other hand, if too much acid is present (low pH), the basic amine nucleophile is completely protonated, so the initial nucleophilic addition step can't occur.

Hydrolysis of imines

The hydrolysis is initiated by protonation of the imine nitrogen resulting in the formation of the iminium ion. After the addition of a water molecule to the carbon of the former imine group, proton transfer is followed from the water molecule to the nitrogen. The whole process is finished by the elimination of an amine and deprotonation to give the neutral aldehyde (Scheme 3).



Scheme 3: The mechanism of imine hydrolysis

Acid is helpful but not an absolute requirement for this reaction. Reasonable mechanisms can be drawn without acid and other counter ions such as Br^-, HSO^{3-}, etc. would work just as well. This is an equilibrium reaction and goes in this direction because of the large excess of water. It is the exact reverse of imine formation. There are certainly other reasonable ways for proton transfer (step 3) and other species besides H_2O that could conceivably act as bases in the last step[52]. The hydrolysis happens even in basic condition; however, it is slower, because of the different mechanism.

3.4 Pro-fragrances

Bioactive volatile organic compounds (VOCs) often serve in nature as signaling compounds for communication between species and are often used as flavor or fragrances in our everyday life. The high volatility of those compounds limits their longevity and their effectivity in long-term use in our everyday life. The effort to eliminate this problem has led to the development of pro-fragrances, which are ideally nonvolatile and odorless fragrance precursors which release the biologically active

VOC by bond cleavage. Only a limited amount of bond cleavage reactions can be used such as hydrolysis, temperature changes and decomposition by light[53], oxygen, enzymes or microorganisms to release the bioactive compounds[54].

Amongst the typical biologically active VOCs released from pro-fragrances belong the alcohols (menthol, citronellol, borneol), aldehydes and ketones (benzaldehyde, cinnamaldehyde, lilal, acetophenone, etc.), esters and lactones (jasmolactone, (*Z*)-3-hexenyl acetate) and other compounds such as limonene or 2,6-dimethylpyrazine[54].

3.4.1 Hydrolysis and the change of the pH value

Water is the most used medium for the most perfumery applications and therefore naturally the hydrolysis, possibly induced by a change in the pH value, may be a suitable trigger to control the release of the VOCs and to achieve increased longevity of the fragrance perception. Representative examples are all kinds of washing methods, in particular, laundry treatments, where the product is stored under acidic (fabric softeners, body lotions, and shampoos) or alkaline conditions (detergents and soaps) before being brought to a neutral pH value at the end of the washing cycle. In fact, most of the literature describing chemical-delivery systems for volatiles is based on the hydrolytic bond cleavage of a broad variety of different precursors[54].

The hydrolysis of imines (carbonyl-amine condensation products) was one of the first reactions that has been described for the release of fragrance and flavor aldehydes and ketones by hydrolysis in an aqueous environment[55]. Schiff bases of aminopropyl polysiloxanes, amino acids, aromatic and polyamines were synthesized for detergents and fabric softeners[56–58] and condensation products with urea, glutamates, and anthranilates were prepared for food applications[55].

Amongst the most used precursors besides imines are carboxylates, such as esters to release alcohols[59] or enol esters to release aldehydes or ketones[60]. Inorganic esters of phosphates, sulfates, sulfites, sulfonates[54], borates[61] as well as aluminates[62], zirconates, and titanates[63] are used too. Besides the above mentioned pro-fragrance forms activated by hydrolysis are silanes, siloxanes[64–66], acetals, ketals[67,68], and other related structures.

3.5 Static headspace-gas chromatography

Headspace analysis refers to the analysis of the gas phase of a binary heterogeneous system in equilibrium. The condensed phase can be liquid or solid. The headspace gas can be investigated by various methods; however, gas chromatography (GC) is ideal for such measurements due to the gaseous phase of the headspace. In headspace-gas chromatography (HSGC) the vapor in contact with the condensed phase in a headspace vial is collected by a needle, transferred into the gas chromatography column and analyzed.

3.5.1 Basic theoretical background of SHGC

As mentioned before, the headspace vial generally contains two phases – the gas phase and the sample (condensed) phase. If the system contains volatile analytes that are soluble in the condensed phase, these will distribute between both phases according to the thermodynamically controlled equilibrium. The system represented by the vial is characterized by the following values:

V_V = total volume of the vial

V_L = volume of the sample (condensed phase)

V_G = volume of the headspace (gas phase)

$$V_V = V_L + V_G \tag{3.3}$$

The relative volumes of the two phases in the vial are characterized by the phase ratio β, representing the ratio of the volumes of the two phases present:

$$\beta = V_G/V_L \tag{3.4}$$

$$\beta = \frac{V_V - V_L}{V_L} = \frac{V_G}{V_V - V_G} \tag{3.5}$$

$$V_G = V_V \cdot \frac{\beta}{1+\beta} \tag{3.6}$$

The amount of the analyte transferred from the sample to the headspace during equilibration is not considered to have any notable change in the volume of the condensed phase. It means that the volume of the original sample V_0 is equal to the volume of the sample phase V_S in any given time.

$$V_0 = V_S \tag{3.7}$$

The initial amount of the analyte in the sample was N_0, and its initial concentration was C_0. After reaching the equilibrium, the amount of the analyte distributed in between the gas and the condensed phase are N_G and N_L and their concentrations are C_G and C_L.

$$C_0 = N_0/V_L \tag{3.8}$$

$$C_L = N_L/V_L \tag{3.9}$$

$$C_G = N_G/V_G \tag{3.10}$$

The distribution of the analyte between the two phases in equilibrium is characterized by the *equilibrium constant* or in GC often used synonymous term *partition* (*distribution*) *coefficient K*. The partition coefficient is dependent on the solubility of the analyte in the condensed phase. Compounds with high solubility will

have a high concentration in the condensed phase compared to the gas phase ($C_L \gg C_G$), so the value of K may be very high. Whereas on the other hand, in the case of analytes with low solubility in the condensed phase, C_L will be close to C_G, or even less than this value, the K will be small. The above-discussed facts can be expressed by the following equations:

$$K = \frac{C_L}{C_G} = \frac{N_L}{V_L} \Big/ \frac{N_G}{V_G} = \frac{N_L}{N_G} \cdot \frac{V_G}{V_L} = \frac{N_L}{N_G} \cdot \beta \tag{3.11}$$

$$N_0 = C_0 \cdot V_L \tag{3.12}$$

$$N_L = C_L \cdot V_L \tag{3.13}$$

$$N_G = C_G \cdot V_G \tag{3.14}$$

$$C_L = K \cdot C_G \tag{3.15}$$

Thus, the following material balance equation can be modified as follows:

$$N_0 = N_L + N_G \tag{3.16}$$

$$C_0 \cdot V_L = C_G \cdot V_G + C_L \cdot V_L = C_G \cdot V_G + K \cdot C_G \cdot V_L = C_G \cdot (K \cdot V_L + V_G) \tag{3.17}$$

Expressing C_0 then C_G:

$$C_0 = C_G \left(\frac{K \cdot V_L}{V_L} + \frac{V_G}{V_L} \right) = C_G (K + \beta) \tag{3.18}$$

$$C_G = \frac{C_0}{K + \beta} \tag{3.19}$$

In a given system under given condition both K and β are constants, thus $(K + \beta)$ and its reciprocals will be constants, too. In other words, in a given system the concentration of the analytes in the headspace is proportional to the original sample concentration. Therefore we can write:

$$C_G = (\text{const}) \cdot C_0 \tag{3.20}$$

It follows from the basic rules of GC that the peak area obtained for a given analyte is proportional to the concentration of the analyte in the analyzed sample. In the case of the HSGC, an aliquot of the headspace is analyzed, in which the concentration of the analyte is C_G. For the obtained peak area A we can write:

$$A = (\text{const}) \cdot C_G \tag{3.21}$$

where the constant includes the influence of a number of analytical parameters and the detector response factor. Combining the two previous equations, we get a new

equation, in which the new constant incorporates the influence of headspace, GC and detector parameters.

$$A = (\text{const}) \cdot C_0 \tag{3.22}$$

The previous equation leads us to two conclusions. The first is that if an aliquot of the headspace at equilibrium is analyzed by GC, the received peak area of the analyte is directly proportional to its concentration in the original sample. The second conclusion is related to the constant in the equation which incorporates the influence of a lot of parameters. Since their numerical evaluation would be very difficult, a prerequisite of reproducible analysis is the exact reproduction of the analytical conditions, and this is true if quantitative measurement is based on the comparative analysis of the sample and standard.

$$A \propto C_{\mathrm{G}} = \frac{C_0}{K + \beta} \tag{3.23}$$

Combining equation and we obtain the equation, which represents the relationship at equilibrium between the peak area A obtained by analyzing an aliquot of the headspace, the concentration of the analyte in the headspace C_{G}, the original sample concentration of the analyte, the partition coefficient K, and β the phase ratio of the vial[69].

3.5.2 Henry's law

Henry's law is one of the gas laws and was formulated by the British chemist, William Henry, in 1803. Henry's law states that the amount of a gas that dissolves in a liquid is directly proportional to the partial pressure of that gas and is mathematically expressed by the equation 3.24.

$$p_{\mathrm{i}} = H_{\mathrm{i}} \cdot C_{\mathrm{i}} \tag{3.24}$$

The subscript i on all the variables refers to the i-th component of a mixture, and p_{i} is the partial pressure of the vapor above the solution, C_{i} is the concentration of component i in the solution, and H_{i} is the Henry's law constant. Typically, the partial pressure would be in pascals, Pa, and the concentration would be in $\mathrm{mol \cdot m^{-3}}$, leading to a Henry's law constant, H_{i}, in $\mathrm{Pa \cdot m^3 \cdot mol^{-1}}$.

Determining the Henry's law constant of VOCs is important to understand their behavior in a system consisting of a liquid (aqueous) phase and a gaseous phase. The higher the value of the Henry's law constant is, the less soluble the VOC in the liquid phase is and the higher its partial pressure p_{i} in the gaseous phase is. In other words, the volatility of the VOC depends on its Henry's law constant; the higher its value is, the more volatile the VOC is.

The determination of the Henry's law constant of VOCs by HSGC is based on the principle that a closed system consisting of a known volume of water, a known

volume of trapped air, and a known amount of VOC attains an equilibrium state where a fraction of the VOC is present in the vapor and the rest is in the water solution. The concentration of the VOC in the gas phase is determined by GC. By multiplying the determined concentration of the VOC by the volume of the headspace we get the amount of the VOC in the gas phase. The remaining amount of VOC is in solution, which can be converted to a concentration in solution by dividing by the volume of the solution. Thus the p_i and C_i for the equilibrated system are determined, which allows us to solve the H_i, the Henry's Law constant[70].

For the quantitative examination of the above described method let's consider a fixed number of moles of a VOC, N_0, added to the closed vial with a liquid volume V_L and a headspace volume V_G. After the equilibrium the distribution of the VOC between the two phases can be expressed by the equation 3.16 and the number of moles in the solution is related to the solution concentration by the relation:

$$N_L = C_L \cdot V_L \tag{3.25}$$

By expressing the concentration of the liquid, C_L, from the combination of the equations 3.16 and 3.25 we obtain equation 3.26.

$$C_L = \frac{N_0 - N_G}{V_L} \tag{3.26}$$

The number of moles in the headspace is given by the ideal gas law:

$$N_G = \frac{pV_G}{RT} \tag{3.27}$$

Substituting equation 3.24 for the partial pressure in equation 3.27 we obtain equation 3.28.

$$N_G = \frac{C_i H_i V_G}{RT} \tag{3.28}$$

Commonly, the terms H_i/RT are grouped to form a unitless version of the Henry's Law coefficient, which is referred to as H_U. Applying this definition on equation 3.28, dividing it by the volume of the headspace, V_L, and applying equation 3.26 for C_i we get:

$$\frac{N_G}{V_G} = \frac{(N_0 - N_G)}{V_L} \cdot H_U = \frac{N_0 H_U}{V_L} - \frac{N_G H_U}{V_L} \tag{3.29}$$

By collecting the terms with N_G and simplifying we get equation 3.30.

$$\frac{N_G}{V_G}\left(1 + H_U \frac{V_G}{V_L}\right) = \frac{N_0 H_U}{V_L} \tag{3.30}$$

Equation 3.30 can be modified into equation 3.31 by multiplying by V_L and dividing by the term in brackets.

$$\frac{N_G}{V_G}V_L = \frac{N_0 H_U}{\left(1 + H_U \frac{V_G}{V_L}\right)} \tag{3.31}$$

The area under the GC signal peak, *S*, is proportional to the concentration of the VOC injected into the GC, thus we have

$$S = k\left(\frac{N_G}{V_G}\right) \tag{3.32}$$

Inserting the equation 3.32 into equation 3.31 we get equation 3.33.

$$SV_L = \frac{kN_0 H_U}{\left(1 + H_U \frac{V_G}{V_L}\right)} \tag{3.33}$$

Equation 3.33 quantitatively expresses the relationship between the GC signal and the ratio of the headspace volume to the liquid volume[70–72].

4 Results and discussion

4.1 Synthesis

The synthesis of the Schiff bases of 6^I-amino-6^I-deoxy-β-cyclodextrin and aldehydes required a four-step routine. The first step was to prepare 6^I-*O*-*p*-toluenesulfonyl-β-cyclodextrin (**1**) from native β-cyclodextrin, then the nucleophilic substitution of the *p*-toluenesulfonyl group with an azido group obtaining 6^I-azido-6^I-deoxy-β-cyclodextrin (**2**), which was later reduced to 6^I-amino-6^I-deoxy-β-cyclodextrin (**3**), which is able to form imines with aldehydes. The individual steps of the synthesis from β-CD to compound **3** are described in the following chapter and are shown in Scheme 4.

4.1.1 6^I-Amino-6^I-deoxy-β-cyclodextrin

6^I-*O*-*p*-Toluenesulfonyl-cyclodextrin

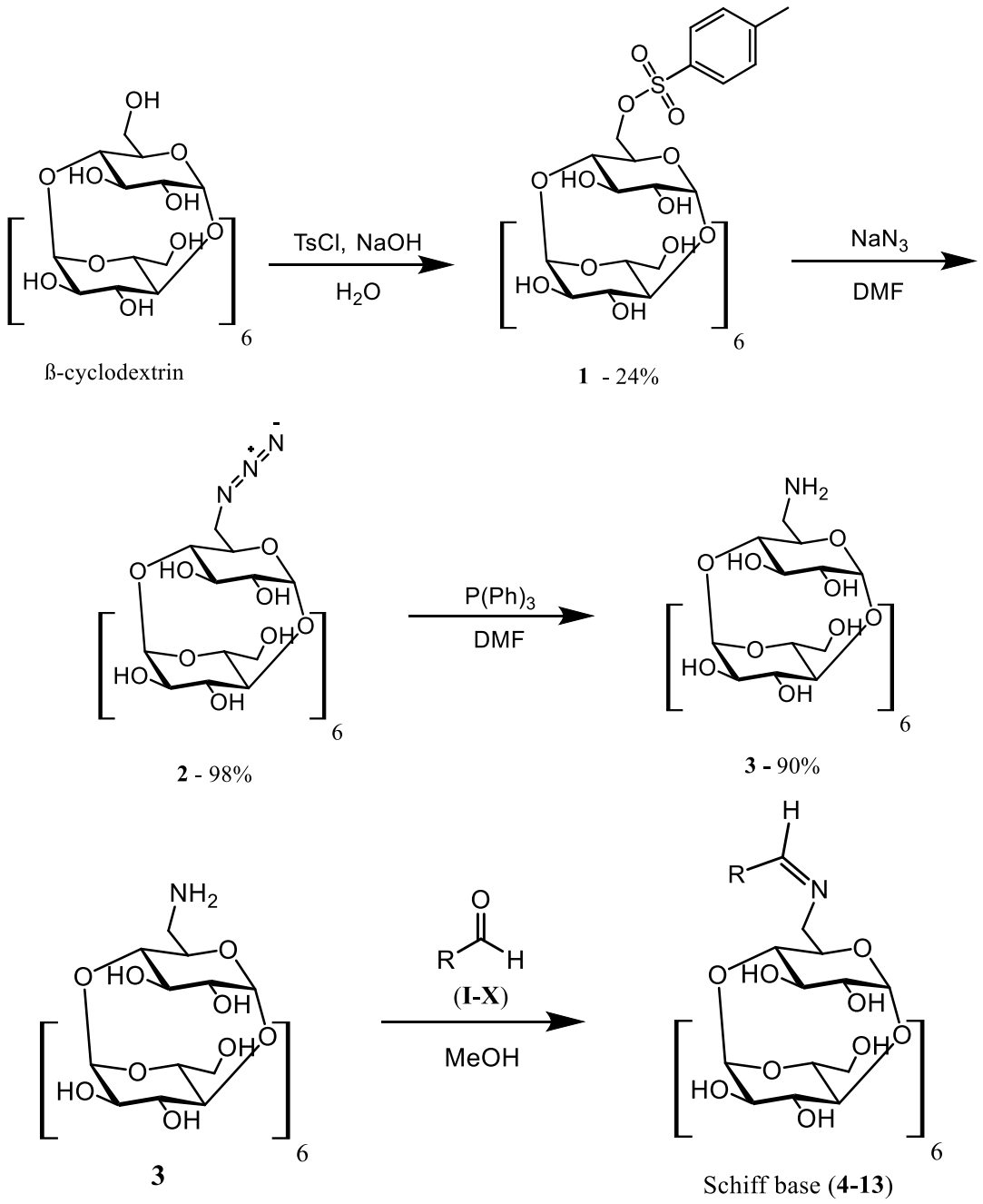
The most commonly used method[42] to prepare the 6^I-*O*-*p*-toluenesulfonyl-β-cyclodextrin (**1**) is the reaction of β-CD with *p*-toluenesulfonyl chloride in the ratio of 1:1.1 in an alkaline aqueous medium. The product was purified by repeated recrystallization (3 times) from 50% methanol.

6^I-Azido-6^I-deoxy-β-cyclodextrin

The preparation of 6^I-azido-6^I-deoxy-β-cyclodextrin (**2**) was carried out by the modification of the published procedure[43] from 6^I-*O*-*p*-toluenesulfonyl-cyclodextrin (**1**) and sodium azide in DMF. The procedure was changed by modification of the purification of the crude product, which was purified on column chromatography with reversed phase silica gel.

6^I-Amino-6^I-deoxy-β-cyclodextrin

By a simple reduction of 6^I-azido-6^I-deoxy-β-cyclodextrin (**2**) with triphenylphosphine in DMF according to the literature[44], 6^I-amino-6^I-deoxy-β-cyclodextrin (**3**) was prepared in quantitative yield. After full conversion, the reaction mixture was poured into acetone, and the excluded precipitate was filtrated and purified on cation exchanger. Lyophilisation was used to acquire the pure product. The 6^I-amino-6^I-deoxy-β-cyclodextrin (**3**) was then used as the starting compound to prepare the Schiff bases with different aldehydes.



Scheme 4: Synthesis of 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin and its Schiff bases with aldehydes

4.1.2 Schiff bases of 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin and aldehydes

Several orientation reactions were set up with 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin (**3**) and cinnamaldehyde to optimize the reaction conditions. Methanol was used as a solvent because it can solubilize (**3**) (5 mg in 1 mL) and aldehydes too. Because during the reaction water molecules are eliminated, which can catalyze the degradation of the synthesized Schiff base; several hygroscopic catalysts were added to the mixture. In six vials 57 mg of (**3**) was suspended in 1 mL of methanol, and 7 µL of *trans*-cinnamaldehyde was added and mixed at room temperature. In five of them 11 mg of $MgSO_4$, $LiClO_4$, $Ca(ClO_4)_2$, triethyl orthoformate and 3Å molecular sieves were added, and one vial was left without hygroscopic catalysts. The reaction was

monitored by TLC with elution mixture EM1 (propanol/water/25% aqueous ammonia/ethyl acetate 6/3/1/1) but because the product was degrading on the TLC sheet (proven by 2D TLC), the Schiff base had to be reduced to the amine using $NaBH_4$ for proper detection. UV detection was used to detect cinnamaldehyde and the Schiff base; ninhydrin was used to detect the amines (also the reduced Schiff base) and 50% H_2SO_4 was used to detect all of the compounds at the same time. The best results were obtained by the reaction, where no catalyst was used.

Because there were troubles with the detection by TLC because the product was hydrolyzing on the TLC sheet and the conversion rate was not clear even after the reduction by $NaBH_4$, the following reactions were monitored only by MS.

The next step was to push the conversion rate to 100%. Compound (**3**) and 5 equivalents of *trans*-cinnamaldehyde (**I**) were solubilized in MeOH and stirred at reflux under argon atmosphere. After 3 hours the conversion rate was around 25%, so another 5 equivalents of *trans*-cinnamaldehyde was added and stirred overnight. The conversion rate was higher but not satisfying so another 30 equivalents of *trans*-cinnamaldehyde was added and after measuring the MS full conversion of (**3**) to 6^I-deoxy-6^I-(3-phenylallylidene)amino-β-cyclodextrin (**4**) was observed. The methanol was distilled off, the crude product was extracted ten times with of hexane and the product was dried on Kugelrohr at 110 °C. The reaction gave yellowish powder (**4**) with the yield of 95.8%. Using this procedure, nine other Schiff bases were prepared (4-13) using the aldehydes (I-X) from Table 2.

It has to be mentioned that some of the aldehydes used in the reactions are stereoisomers containing *R*/*S* or *cis*-*trans* isomers. The cinnamaldehyde used in the reactions was *trans*-cinnamaldehyde (**I**), the citral (**IX**) was a mixture of *cis* and *trans* isomers and the cyclamen aldehyde (**II**) and lilial (**III**) were racemic mixtures enantiomers. This means that the Schiff bases prepared form citral, cyclamen aldehyde and lilial are diastereomers and basically there were two products obtained in all of the three cases. Those products were not separated mostly because it the case of pro-fragrances it is not playing a role in the releasing of the VOCs. The separation would also be difficult because the products are very similar and unstable.

The detailed procedures of the imine derivatives of β-cyclodextrin synthesis are shown in chapter 5.2.2. Table 3 summarizes the obtained products from the synthesis of Schiff bases of 6^I-amino-6^I-deoxy-β-cyclodextrin and the selected aldehydes. The final imines were then analyzed and the release of the VOCs was studied (chapters 4.3 and 4.4).

Table 2: The list of aldehydes used in the synthesis of Schiff bases

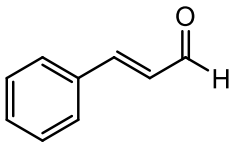
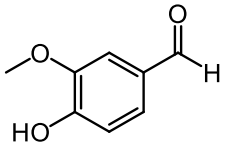
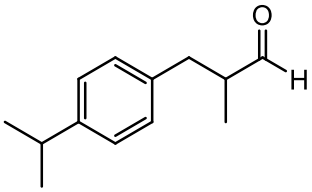
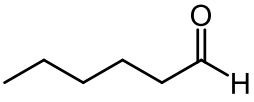
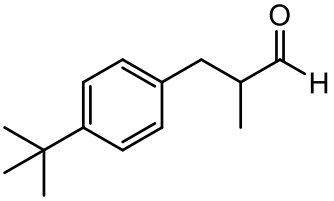
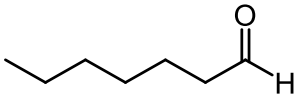
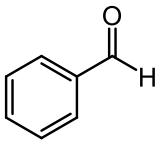
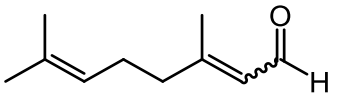
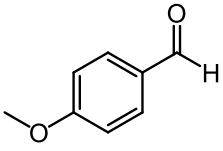
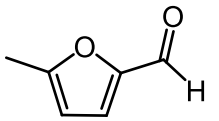
Number	Aldehyde	Number	Aldehyde
I	 *trans*-cinnamaldehyde	VI	 4-hydroxy-3-methoxybenzaldehyde vanillin
II	 3-(4-isopropylphenyl)-2-methylpropanal cyclamen aldehyde	VII	 hexanal
III	 3-(4-(*tert*-butyl)phenyl)-2-methylpropanal lilial	VIII	 heptanal
IV	 benzaldehyde	IX	 3,7-dimethylocta-2,6-dienal citral
V	 4-methoxybenzaldehyde anisladehyde	X	 5-methylfuran-2-carbaldehyde 5-methylfurfural

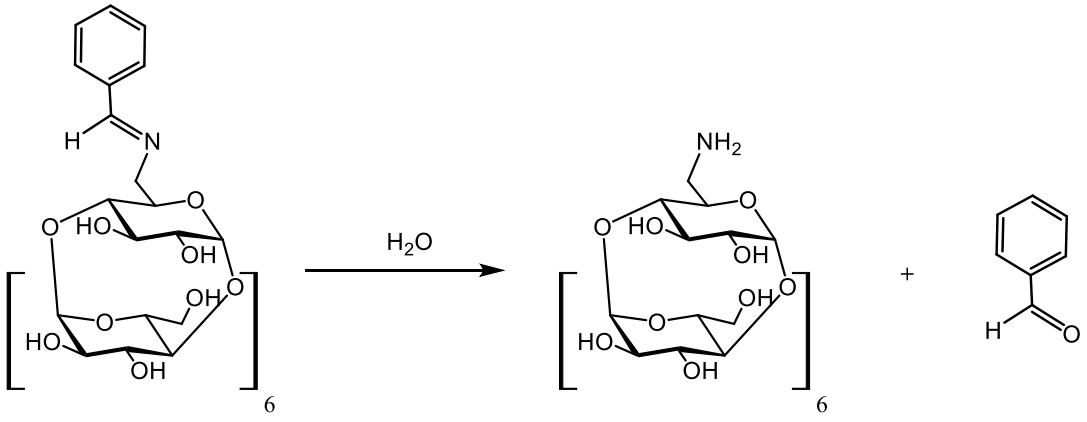
Table 3: Schiff bases of 6^I-amino-6^I-deoxy-β-cyclodextrin and the selected aldehydes.

Aldehyde	Product	Yield (%)	Mixture of isomers
I	6^I-deoxy-6^I-(3-phenylallylidene)amino-β-cyclodextrin (**4**)	96	no
II	6^I-deoxy-6^I-((3-(4-isopropylphenyl)-2-methylpropylidene)amino)-β-cyclodextrin (5)	83	yes
III	6^I-((3-(4-(*tert*-butyl)phenyl)-2-methylpropylidene)amino)- 6^I-deoxy-β-cyclodextrin (**6**)	91	yes
IV	6^I-benzylideneamino-6^I-deoxy-β-cyclodextrin (**7**)	86	no
V	6^I-deoxy-β-6^I-(4-methoxybenzylidene)amino-β-cyclodextrin (**8**)	82	no
VI	6^I-deoxy-6^I-(4-hydroxy-3-methoxybenzylidene)amino-β-cyclodextrin (**9**)	96	no
VII	6^I-deoxy-6^I-hexylideneamino-β-cyclodextrin (**10**)	?	no
VIII	6^I-deoxy-6^I-heptylideneamino-β-cyclodextrin (**11**)	97	no
IX	6^I-deoxy-6^I-(3,7-dimethylocta-2,6-dien-1-ylidene)amino-β-cyclodextrin (**12**)	80	yes
X	6^I-deoxy-6^I-((5-methyltetrahydrofuran-2-yl)methylene)amino-β-cyclodextrin (**13**)	83	no

4.2 Kinetic studies of the imines hydrolysis by NMR

For the kinetic studies of the hydrolysis of imino-CDs, 6^I-benzylideneamino-6^I-deoxy-β-cyclodextrin (**7**) was chosen. The release of the benzaldehyde was studied by ^{1}H NMR spectroscopy. Aqueous 0.1M phosphate buffer solutions of pH 1.08, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00, 10.00 11.00, 12.00 and 12.80 were prepared according to protocols (see chapter 5.1.) using deuterium oxide instead of distilled water to facilitate NMR spectroscopy experiments. Because of the bad solubility of the pro-fragrance in water, it had to be dissolved in deuterated dimethyl sulfoxide and then just before starting the measurements the buffer solutions were added and mixed in ratio 1/1. The samples were kept at ambient temperature (20–25 °C).

Every measurement for every pH value was repeated at least three times with about 10 mg of pro-fragrance (**7**). The intervals of the measurements of the benzaldehyde release by ^{1}H NMR spectroscopy were carried out in 2 minute to 24 hour intervals for several days for the buffers with pH values from 1.14 to 4.00 and for the rest of the buffers the intervals were from 2 hours to several days for up to 3 months (for basic pH). The integrals of the appearing signal at 8.30 ppm, corresponding to the hydrogen of the imine group of the non-hydrolyzed Schiff base, were compared to the integral of the signal corresponding to the same proton of the pro-fragrance measured in DMSO-d_6 (without buffer) used as a blank. The scheme of the hydrolysis, where the benzaldehyde is released and the 6^I-amino-6^I-deoxy-β-cyclodextrin regenerated is showed in Scheme 5.



(**7**) (**3**)

Scheme 5: Hydrolysis of 6^I-benzylideneamino-6^I-deoxy-β-cyclodextrin (7)

The experiments were conducted in a deuterated solvent so that ^{1}H NMR spectroscopy could be used to monitor the extent of released benzaldehyde in situ. We note that the experiment was conducted in a closed system with a 50% content of DMSO-d_6 to enable dissolution of the pro-fragrance, which influenced the equilibrium between dissociation and formation reactions. Figure 4 shows an example of the change of the integral of the peak at 8.30 ppm in the acquired ^{1}H NMR spectrum at pH 9 over the course of 20 days.

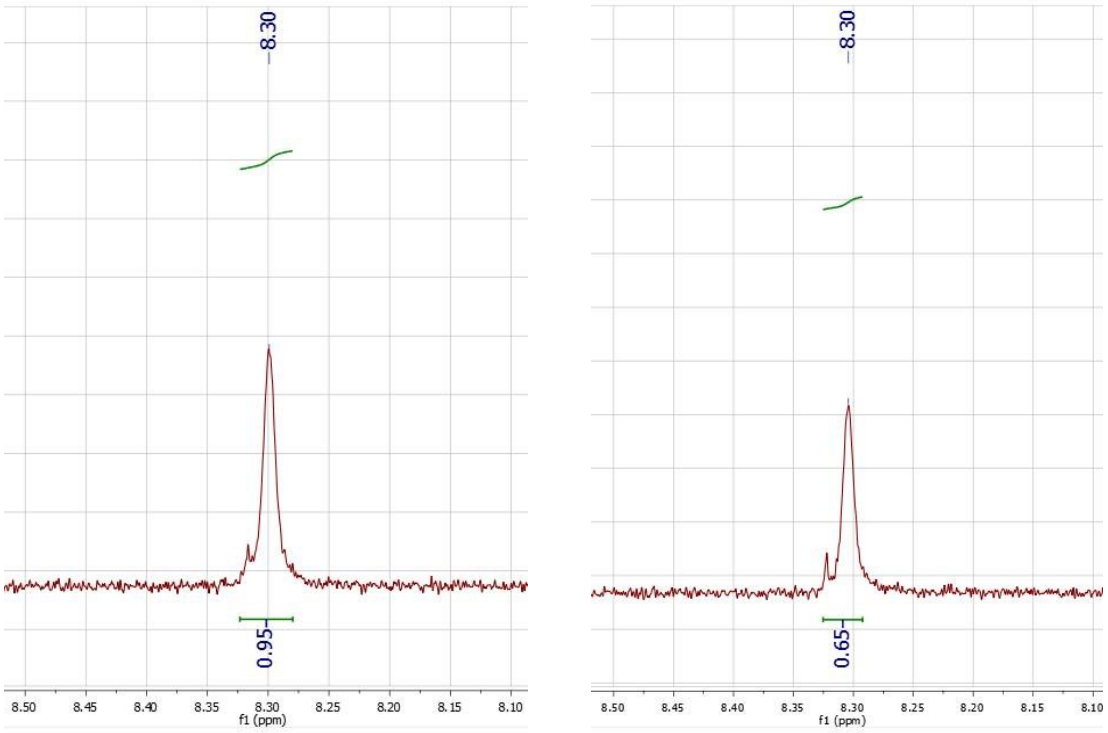


Figure 4: The change of the integral of the peak at 8.30 ppm in the acquired ^{1}H NMR spectrum at pH 9 over the course of 20 days.

Using the program OriginPro 2017, 10 plots were built from the obtained data showing the dependency of the hydrolysis of the imine depending on the pH of the solution prom pH 12.80 to 4.00 (Figure 5 and Figure 6). The obtained data points were fit with several types of functions such as mono-exponential or double-exponential functions, however the fittings was not perfect in every case. The use of the mono-exponential function was chosen (equation 4.1), because decomposition reactions, such as hydrolysis with a high excess of water, are mostly first order reactions which have a mono-exponential decay. Other fittings such as double-exponential functions were not good in this case. The hydrolysis for pH 1.08, 2.00 and 3.00 was too fast to investigate it by ^{1}H NMR spectroscopy.

In equation 4.1 I_0 is the intensity of the imine peak of the blank, I represents the intensity of the measured imine peak, t_0 is the starting time of the experiment, t is the time of the measurement of the imine peak, A is a constant and τ is the time constant.

$$I = I_0 + Ae^{\frac{-(t-t_0)}{\tau}} \tag{4.1}$$

$$t_{1/2} = \tau \cdot \ln 2 \tag{4.2}$$

From the equations of the mono-exponential fittings the time constants of the hydrolysis, τ, were obtained. Using equation 4.2, the halftime of the hydrolysis, $t_{1/2}$, was calculated from the time constant (Table 4). The time constants and the halftimes of the hydrolysis for each pH are shown in Figure 7.

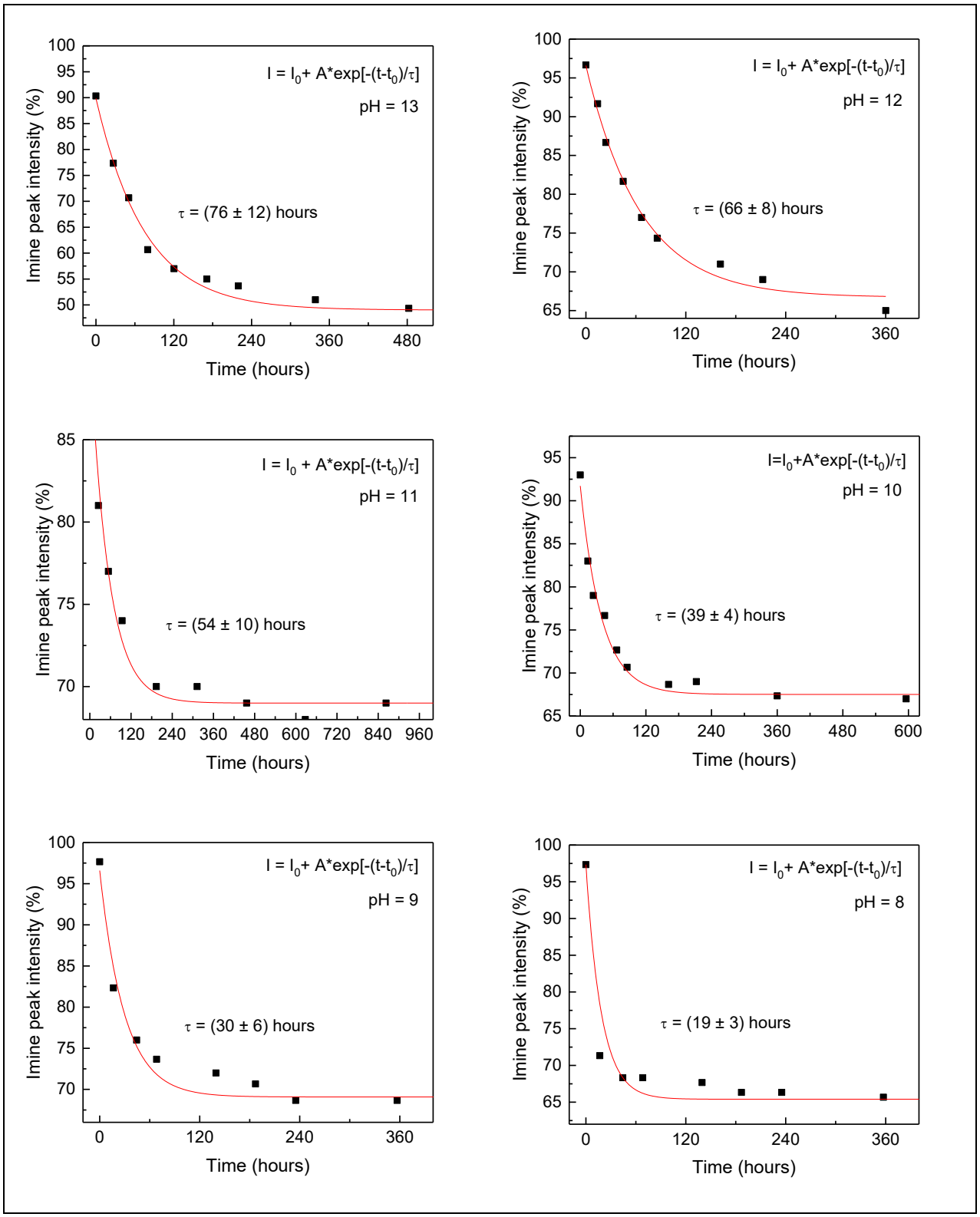


Figure 5: The course of the hydrolysis of 6^{I}-benzylideneamino-6^{I}-deoxy-β-cyclodextrin (7) in buffers from pH 12.80 to 8.00

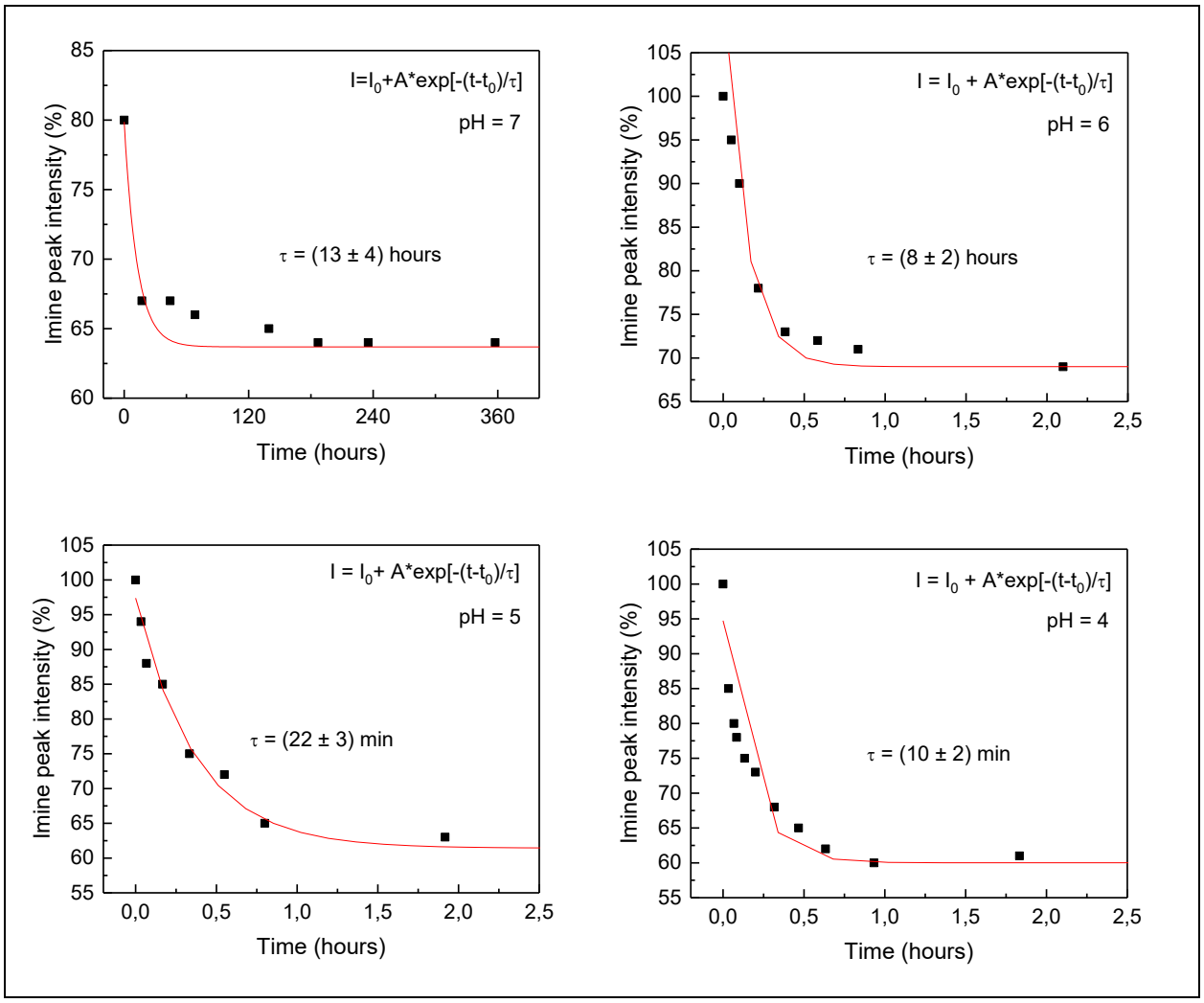


Figure 6: The course of the hydrolysis of 6^{I}-benzylideneamino-6^{I}-deoxy-β-cyclodextrin (7) in buffers from pH 7.00 to 4.00

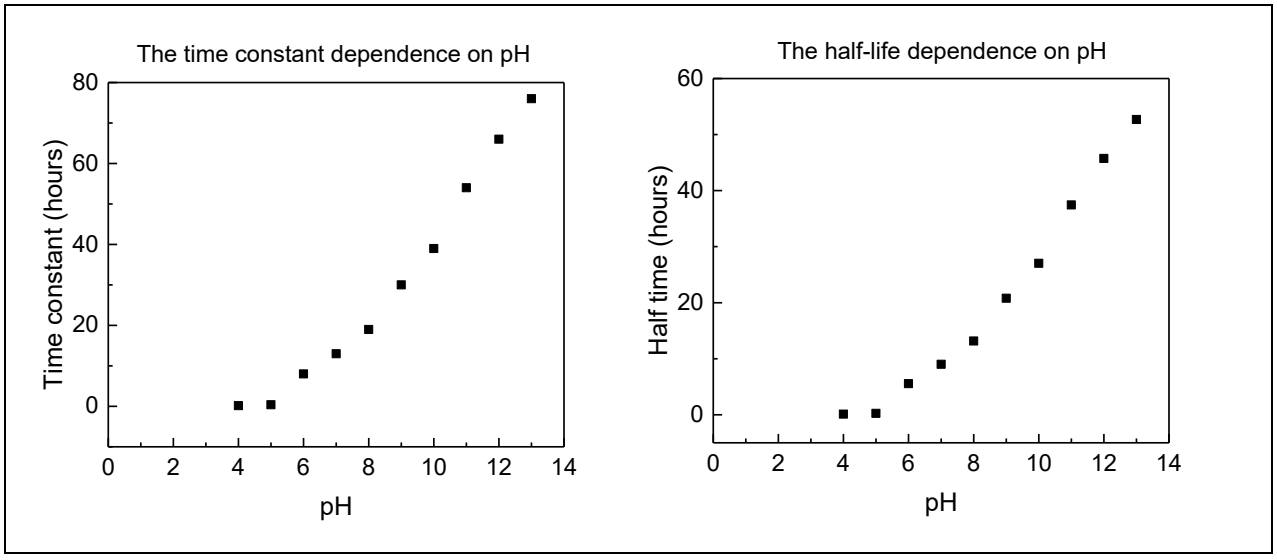


Figure 7: The dependency of the time constants, τ, and the halftimes, $t_{1/2}$ on pH

Table 4: The time constants, τ, and the halftimes, $t_{1/2}$, of the hydrolysis for each pH

pH	12.80	12.00	11.00	10.00	9.00
τ (h)	76 ± 12	66 ± 8	54 ± 10	39 ± 4	30 ± 6
$t_{1/2}$ (h)	53 ± 8	46 ± 6	37 ± 7	27 ± 3	21 ± 4
pH	8.00	7.00	6.00	5.00	4.00
τ (h)	19 ± 3	13 ± 4	8 ± 2	0.37 ± 0.05	0.17 ± 0.03
$t_{1/2}$ (h)	13 ± 2	9 ± 3	6 ± 1	0.26 ± 0.03	0.12 ± 0.02

A strong difference is seen between acidic conditions, under which the imino-β-CD is unstable and the benzaldehyde releases fast over the time course investigated, and basic conditions, under which the benzaldehyde is released slowly. In all cases equilibrium between the hydrolyzed and the non-hydrolyzed pro-fragrance is obtained – the more acidic the solution is, the equilibrium is reached faster and is hydrolyzed to a higher degree. This is consistent with the mechanism for the release-step,

It has to be noted that during the NMR studies no degradation of the cyclodextrin was detected with the available methods and at pH of 11.00, 12.00 and 12.80 a decrease and later, in the case of pH 12.80, a total disappearing of the hydrogen peak of the aldehyde group of benzaldehyde at 10.06 ppm was observed due to Cannizaro's reaction. The benzaldehyde at pH 12.80 was fully converted to benzyl alcohol and benzoic acid after 5 days.

4.3 Static headspace-gas chromatography experiments

Cyclodextrins are used as solubilizers and/or sustained-release carriers for flavor and fragrance material in aqueous solution in the cosmetic, food, and pharmaceutical fields as they can modify the aroma release and flavor perception. In the case of this thesis, the aromas are covalently bound to the rim of the cyclodextrin with an imine bond creating a pro-fragrance. To study the release of the aroma from the pro-fragrance at different conditions, the behavior of the native β-CD and the aroma alone had to be studied. Four aldehydes were chosen for the analysis including benzaldehyde, the one which was used for the NMR studies; 5-methylfurfural including an oxygen heteroatom; cinnamaldehyde, with an aromatic ring on an aliphatic chain and finally heptanal, an aliphatic aldehyde. Later the release of the aromas from the Schiff bases was studied by multiple headspace-gas chromatography. All the experiments with SHGC and MHGC were carried out under the supervision of Dr. Sophie Fourmentin Lamotte from the department of environmental chemistry (Unité de Chimie Environnementale et Interactions sur le Vivant – UCEIV) at the university of Dunkirk in France (Université du Littoral Côte d'Opale – ULCO).

4.3.1 The formation constants of β-CD and selected aldehydes

To analyze the release of the VOCs from the prepared imino-β-CDs, firstly a modified method using static headspace-gas chromatography (SHGC) was used to determine the formation constant (stability constant), K_f, of the inclusion complexes formed between the pure β-CD and the aroma. The saturation of the headspace-gas area was measured and after the complexation behavior of β-CD for benzaldehyde, 5-methylfurfural, cinnamaldehyde and heptanal were investigated in aqueous solution by SHGC. The detailed description of the experiments is in chapter 5.1.

Calibration curves of the selected aldehydes

First, the saturation of the headspace-gas area had to be measured, which shows the maximum concentration (in ppm) of the aldehydes that can be used in the following experiments. According to Table 5, five (or six) 22 mL headspace vials containing 10 mL solutions with different concentrations of aroma were prepared for each studied aldehyde. After the equilibrium, the headspace-gas was analyzed by SHGC using the method described above. Using the areas of the analytes obtained from the SHGC and the concentration of each solution, a calibration curve was set. The saturation of the headspace-gas area was reached, where the curvature of the graph was no longer linear. The results are recorded in Table 5 and the calibration curves are shown in Figure 8. From the calibration curves for each aldehyde parameters of the linear dependence of the the area of the GC peak, A, on the concentration of the aldehyde, [G], was obtained. These equations can be used to calculate the concentration of the aldehyde in the solution using the value of the area of the GC peak. The calibration curves and the equations are Figure 8. All the experiments were done three times.

Table 5: The measurement of the saturation of the headspace-gas area and the maximal saturation for each aldehyde

	Sample	1	2	3	4	5	6	Maximal saturation (ppm)
Concentration (ppm)	Benzaldehyde	20	50	100	200	500	—	200
	5-Mehylfurfural	20	50	100	200	500	—	200
	Cinnamaldehyde	100	200	350	500	700	900	700
	Heptanal	5	10	20	50	100	150	50

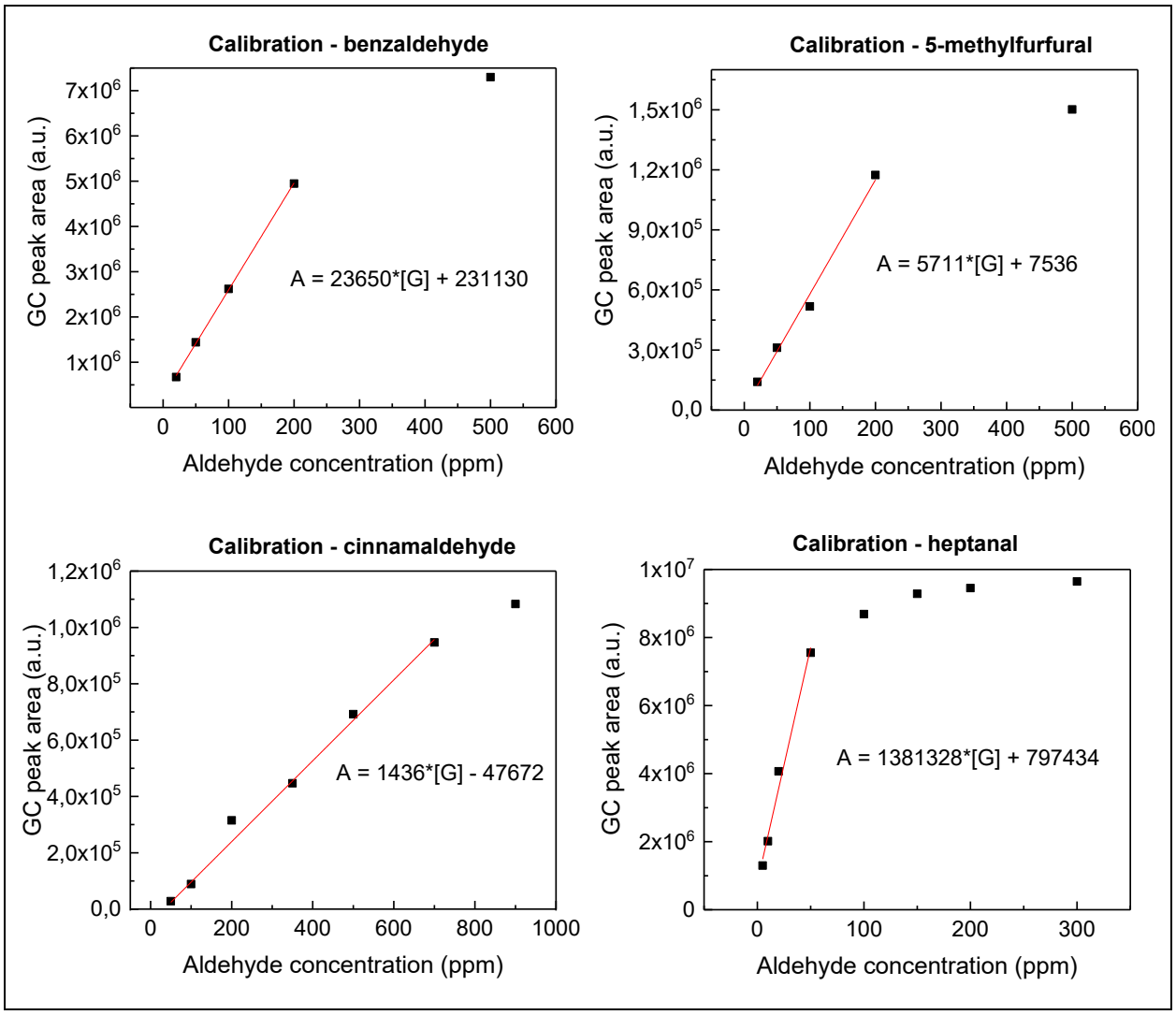


Figure 8: The calibration curves and the equations for the aldehydes

The formation constants of β-CD and selected aldehydes

Because of the limited time, four aldehydes were chosen for this experiment. The host/guest system was studied by a SHGC titration method developed in the laboratory of Dr. Sophie Fourmentin Lamotte for volatile organic compounds[73–75]. Different concentrations of β-CD were used at constant guest (G) concentration. Assuming 1:1 ratio binding, the total concentration of guest in aqueous solution ($[G]_0$) and the total CD concentration ($[CD]_0$) were expressed in equations 4.1 and 4.2, where [CD/G] is the concentration of the associated complex. The $[G]_0$ after equilibrium was determined by subtracting the number of moles of guest in the gaseous phase.

$$[G]_0 = [G] + [CD/G] \quad (4.3)$$

$$[CD]_0 = [CD] + [CD/G] \quad (4.4)$$

Then, in the presence of CD, the peak area can be expressed as in equation 4.5, with A the integrated area counts of the GC peak for a given sample, and α, a specific parameter of the headspace.

$$A = \alpha([\mathrm{G}]_0 - [\mathrm{CD/G}]) \tag{4.5}$$

The formation constant (equation 3.2) can be written as follows:

$$K_\mathrm{f} = \frac{[\mathrm{CD/G}]}{[\mathrm{G}][\mathrm{CD}]} = \frac{[\mathrm{CD/G}]}{([\mathrm{G}]_0 - [\mathrm{CD/G}]) \cdot ([\mathrm{CD}]_0 - [\mathrm{CD/G}])} \tag{4.6}$$

Thus, [CD/G] can be estimated by:

$$[\mathrm{CD/G}] = -\frac{1}{2}\sqrt{\left(\frac{1}{K_\mathrm{f}} + [\mathrm{CD}]_0 + [\mathrm{G}]_0\right)^2 - 4[\mathrm{CD}]_0[\mathrm{G}]_0} + \frac{1}{2}\left(\frac{1}{K_\mathrm{f}} + [\mathrm{CD}]_0 + [\mathrm{G}]_0\right) \tag{4.7}$$

For a given value of K_f, [CD/G] was known, and thus a theoretical value was calculated for the peak area. An algorithmic treatment was then applied to minimize the difference between the experimental and theoretical values of the peak area leading to the adequate formation constant[76].

The experiments for the determinations of the formation constant were done at 30 °C in 22 mL headspace vials containing 10 mL solutions of β-CD with four concentrations (1 mM, 4 mM, 7 mM and 10 mM) and two blank vials without β-CD. Then the aldehydes were added to the prepared vials to obtain a 100 ppm solution of benzaldehyde, 200 ppm solution of 5-methylfurfural, 400 ppm solution of cinnamaldehyde and 20 ppm solution of heptanal. The vials were sealed using silicone septa and aluminium foil, and after 30 minutes of equilibrium, the headspace gas was analyzed. The experiments were provided 3 times. From the obtained data using equation 4.4 the formation constants were calculated (Table 6). Representation of the experimental points obtained for benzaldehyde and β-CD is shown in Figure 9.

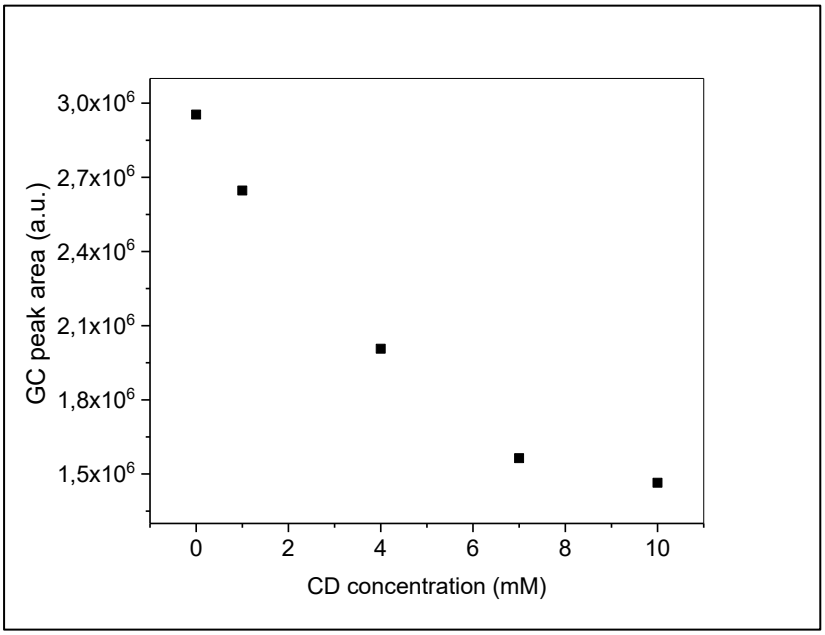


Figure 9: The dependence of the area of the GC peak of benzaldehyde on the concentration of β-CD

Table 6: The experimental values of the K_f for β-CD and the selected aldehydes

	benzaldehyde	5-methylfurfural	cinnamaldehyde	heptanal
K_f (M^{-1})	103	61	162	452

4.3.2 The determination of Henry's law constants

The determination of Henry's law constant for the aldehydes was important, because it reflects their behavior in a system consisting of an aqueous phase and a gaseous phase. The higher the value of the Henry's law constant is, the less soluble the aldehyde in the aqueous phase is and the higher its partial pressure pi in the gaseous phase is. In other words, the higher the Henry's Law coefficient is, the more volatile the aldehyde To determine the Henry's Law coefficient, an experiment was designed, where a number of vials were prepared, each having a different ratio of water volume to headspace volume and a small amount of 10000 ppm aldehyde solution in ethanol was added to each vial (Table 6). The headspace gas was then chromatographed. The GC signal is then proportional to the concentration of the VOC in the headspace, and the experimental data can be fit to equation 3.33, and the Henry's law coefficient can be extracted.

Examination of equation 3.33 shows that when the product of $H_U(V_G/V_L)$ is small compared to unity, the product SV_L is constant, independent of the headspace to liquid volume ratio. In this case, the value of H_U cannot be accurately determined. Similarly, for large values of $H_U(V_G/V_L)$, S is proportional to $1/V_G$, and shows no sensitivity to the value of H_U. These two limiting cases demonstrate that the best values of V_G/V_L to determine the Henry's law coefficient for a given VOC occurs when the product $H_U(V_G/V_L)$ is close to unity.

$$SV_L = \frac{kN_0H_U}{\left(1 + H_U\frac{V_G}{V_L}\right)} \tag{3.33}$$

For ease of analysis equation 3.33 can be transformed into a more tractable form. By the experimental design, the product kN_0 was fixed. Equation 3.33 can then be transformed to linear expression with the independent variable V_G/V_L.

$$\frac{1}{SV_L} = \frac{1}{kN_0}\left(\frac{V_G}{V_L}\right) + \frac{1}{kN_0H_U} \tag{4.8}$$

Thus, a plot of $1/SV_L$ versus V_G/V_L should be linear and the unitless Henry's law coefficient is given by the ratio of the slope divided by the intercept. The final values of Henry's law constants for the examined aldehydes are in Table 7.

Table 7: Henry's law constant experiment and results

	Vial number	benzaldehyde	5-methylfurfural	cinnamaldehyde	heptanal
Volume of water (µL)	1	100	100	100	100
	2	200	200	200	200
	3	300	300	300	300
	4	500	500	500	500
	5	700	700	700	700
	6	1000	1000	1000	1000
	7	—	—	1500	1500
	8	—	—	2000	2000
	9	—	—	2500	—
	10	—	—	3000	—
Volume of 10000 ppm aldehyde solution (µL)		50	50	50	5
Henry's law constant H_U ( )		0.1001	0.0204	0.0040	0.1648

The conclusion of this experiment is that the most volatile aldehyde used in the experiments is heptanal with the value of the unitless Henry's law constant 0.1648, following with benzaldehyde (H_U = 0.1001) and 5-methylfurfural (H_U = 0.0204) and the least volatile is cinnamaldehyde (H_U = 0.0040).

4.3.3 The effect of Cannizaro's reaction on benzaldehyde at pH 11

As observed in the ^{1}H NMR experiments, at pH 11 and 12 and 12.80, Cannizaro's reaction takes place and the benzaldehyde in the solution is slowly converted to benzyl alcohol and benzoic acid and in the case of pH 12.80, the aldehyde disappears after 5 days. Because the release of the aldehydes was studied at pH 11, too, the effect of the cannizaro's reaction had to be studied with SHGC likewise.

A 50 ppm solution of benzaldehyde in 0.1M aqueous phosphate buffer of pH 11 was prepared and the headspace-gas was analyzed during 40 days. The results of the experiment are shown in Figure 10.

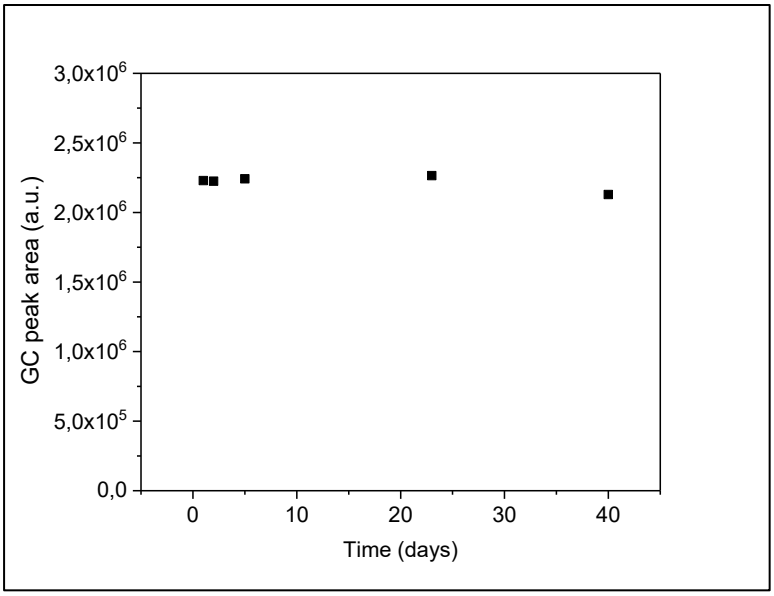


Figure 10: The effect of Cannizaro's reaction on benzaldehyde at pH 11 during 40 days

As it can be seen on Figure 10, Cannizaro's reaction doesn't have any significant effect on the release of the aldehyde from the 0,1M aqueous phosphate buffer of pH 11. This is probably due to the fact that benzaldehyde is much less soluble in the aqueous phosphate buffer used in the SHGC experiment. In the case of 0.1M aqueous phosphate buffer solutions of pH 11 in deuterium oxide and DMSO-6d in ratio 1:1 used for the ^{1}H NMR experiments, the aldehyde is fully solubilized and is more exposed to hydroxyl anions, so the degradation of the aldehyde can take place.

4.4 Multiple headspace-gas chromatography experiments

The dynamic release of aldehydes from the imino-β-CDs was studied by multiple headspace extraction (MHE). In principle, MHE is a stepwise headspace extraction for the quantitative analysis of volatiles in samples. At each step, equilibrium conditions are established in the vial between the sample and its gas phase of the vial. The concentration of the analyte in the gas phase of the vial decreases during the series of extraction steps. Thus, the sum of the amounts of the analyte removed in the individual extractions will be equal to the total amount of analyte present in the original sample[69].

Before starting the MHE of the imino-β-CDs, the MHE of the aldehydes alone had to be performed to use it as a blank for comparison. First, the effect of the pH of the 0.1M aqueous phosphate buffers was investigated at pH 2.00, 5.00, 7.00, 9.00 and 11.00, later, the effect of humidity in the solid state was investigated at 0%, 11%, 48% and 97% humidity.

A calibration curve was set for benzaldehyde to examine the effect of the pH of the phosphate buffer on the volatility of the compound. Aqueous 10 mL of 0,1M

phosphate buffers of pH 2.00, 5.00, 7.00, 9.00 and 11.00 were prepared with the concentration of 20, 50, 100 and 200 ppm of benzaldehyde. A blank experiment was set up using distilled water, too. The vials were measured on SHGC using the method described in chapter 5.1. The results are shown in Figure 11.

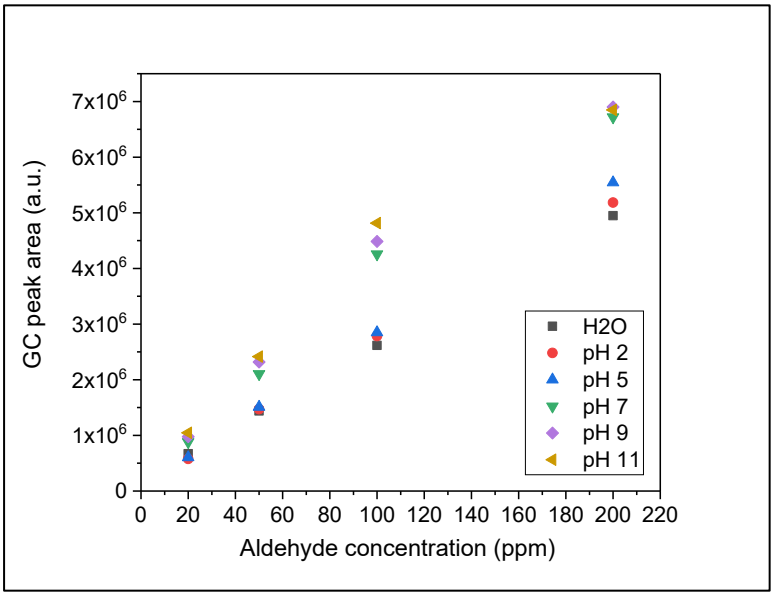


Figure 11: The effect of the pH of the solution on the volatility of benzaldehyde

As seen on Figure 11, the more acidic the solution is, the volatility of the benzaldehyde is lower, in basic solutions the benzaldehyde is slightly more volatile. This phenomenon happens due to the formation of more hydrogen bonds in the acidic solutions than in basic. The benzaldehyde in blank experiment with distilled water at higher concentrations is less volatile than in the buffer at pH 2. This happens because of the fact that there are no ions in high concentrations as in the 0.1M buffers, which have the tendency to make the benzaldehyde more volatile due to their high ion concentration.

4.4.1 Multiple headspace-gas extraction of the aldehydes

The behavior of three of the aldehydes – benzaldehyde, 5-methylfurfural and heptanal – was investigated by MHE in 10 mL of 0,1M aqueous phosphate buffers of pH 2.00, 5.00, 7.00, 9.00 and 11.00 for benzaldehyde and 2.00, 7.00 and 11.00 for heptanal and 5-methylfururral.

Into 10 mL of phosphate buffers benzaldehyde and 5-methylfurfural was added to obtain the concentration of 50 ppm and heptanal to obtain the concentration of 10 ppm. The samples were then measured on SHGC using MHE using the method described in chapter 5.1 with the change that there is no equilibrium time in the beginning. MHE was performed first nine times in 4 minute intervals, nine times in 10 minute intervals and 9 times in 60 minute intervals for benzaldehyde and 5-methylfurfural and for heptanal the 4 minute intervals were changed to 5 because of its longer retention time. The final results are shown in Figure 12.

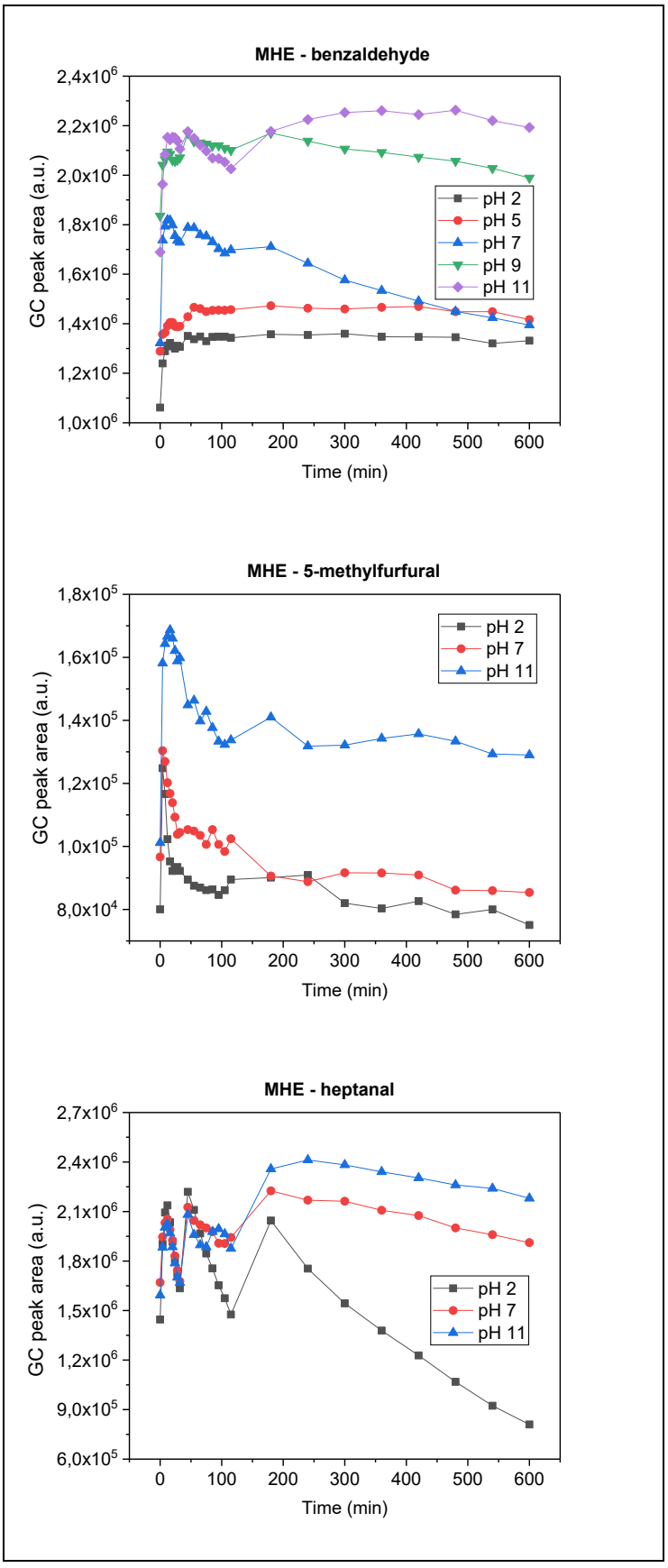


Figure 12: MHE of benzaldehyde, 5-methylfurfural and heptanal in 0.1 M aqueous phosphate buffers at different pH

As visible on the in Figure 12, in every case in the beginning when the extraction takes place every 4 or 5 minutes, the aldehyde is coming fast from the solution to the headspace in big amounts. Later, when the headspace-gas is analyzed every 10 minutes, the aldehyde starts to decrease, however it has more time to obtain an equilibrium state then before. When the one hour extraction interval comes, there is usually a higher concentration of the aroma in the headspace-gas because the time to obtain the equilibrium is even longer, but after that by more extractions a decrease of the headspace-gas aroma concentration is observed. That means that the concentration of the aldehyde in the solution is starting to lower and there is no more source of the aldehyde refill the headspace-gas to the same concentration as before. The volatility of the aldehydes shows the same trends as in Figure 14. The more basic the solution is, the more volatile the aldehyde is.

4.4.2 Multiple headspace-gas extraction of aldehydes from imines at different pH

For this experiment three prepared Schiff bases were used because of the limited time – one aromatic, one aliphatic and one with a heterocycle. The release of aldehydes from three prepared imino-β-CD – 6^{I}-benzylideneamino-6^{I}-deoxy-β-cyclodextrin (**7**), 6^{I}-deoxy-6^{I}-((5-methyltetrahydrofuran-2-yl)methylene)amino-β-cyclodextrin (**13**) and 6^{I}-deoxy-6^{I}-heptylideneamino-β-cyclodextrin (**11**) – was investigated by MHE in 10 mL of 0,1M aqueous phosphate buffers of pH 2.00, 5.00, 7.00, 9.00 and 11.00 compound (**7**) and 2.00, 7.00 and 11.00 for compounds (**13**) and (**11**).

Into 10 mL of 0,1M aqueous phosphate buffers approximately 4.10 mg of compound (**7**), (**13**) and (**11**) was added, sealed using silicone septa and aluminium foil and mixed. The samples were then immediately measured on SHGC using MHE using the method described in chapter 5.1 with the change that there is no equilibrium time in the beginning. MHE was performed first nine times in 4 minute intervals, nine times in 10 minute intervals and 9 times in 60 minute intervals for the release of benzaldehyde from compound (**7**) and 5-methylfurfural from compound (**13**) and for the release heptanal from compound (**11**) the 4 minute intervals were changed to 5 because of its longer retention time. The final results are shown in Figure 13.

As visible on the in Figure 13, in every case in the beginning when the extraction takes place every 4 or 5 minutes, the hydrolysis of the imine bond starts and the aldehyde is being released from the solution to the headspace-gas area. In the case of the imino-β-CD (**13**) and (**7**) – at pH 5.00 and 7.00 a fast release of aldehyde occurs. This can be explained also as the release of the remained aldehyde from the cyclodextrin cavity which remained after the separation and was released from the higher temperature (see chapter 4.4.3) Later, when the headspace-gas is analyzed every 10 minutes, the aldehyde starts to obtain a more steady state and the plot gets more horizontal. When the one hour extraction interval comes, there is usually a higher concentration of the aroma in the headspace-gas because the time to obtain the equilibrium is even longer, and more imine bonds have time to hydrolyze. This is the most visible in the case of the release of heptanal from compound (**11**).

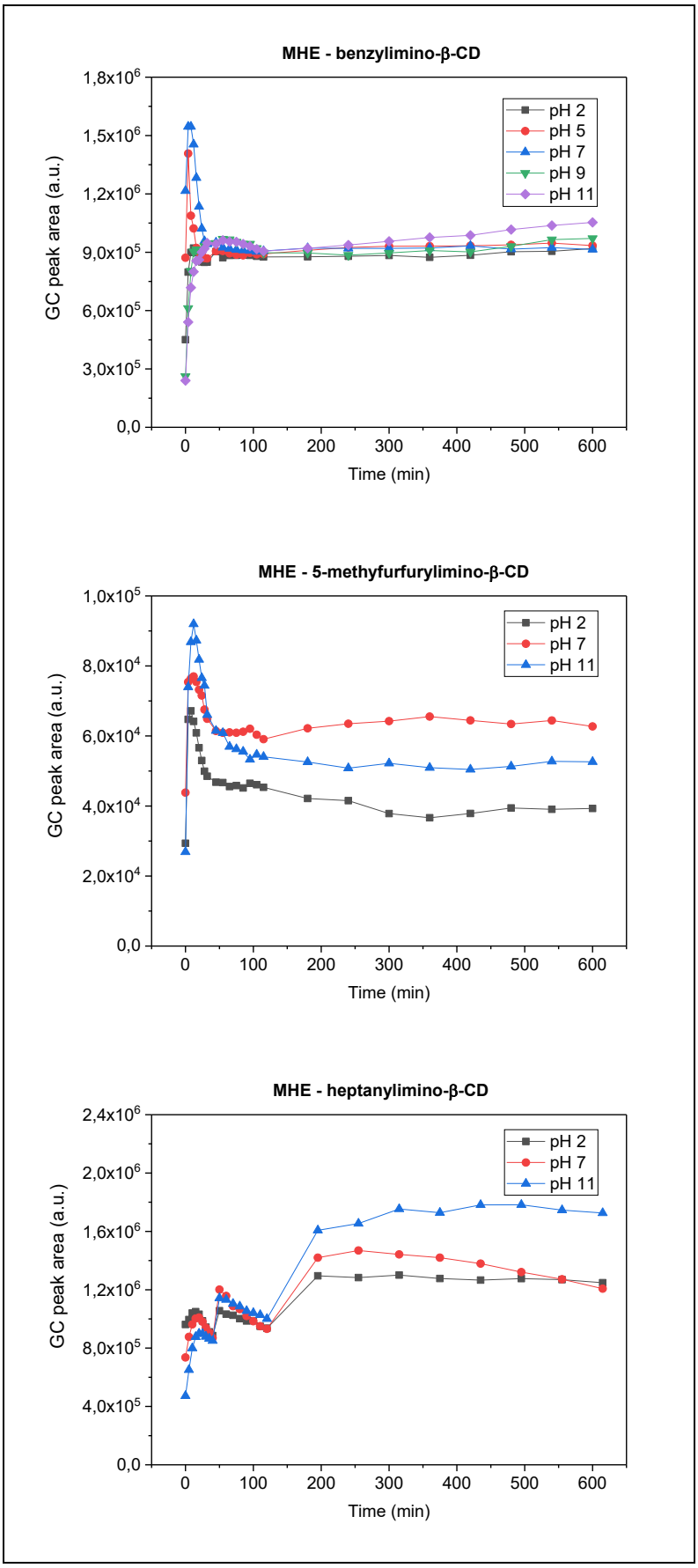


Figure 13: MHE of Schiff bases 7, 13 and 11 at in 0.1 M aqueous phosphate buffers at different pH

After this the release process reaches a steady state in which the imine bonds are hydrolyzed and the aldehydes are slowly released and transferred to the headspace-gas. That means that the concentration of the aldehyde in the headspace-gas is not starting to lower as in the case of the free aldehyde release (Figure 12), because there is always more source of the aldehyde to refill the headspace-gas to the same concentration as before.

The volatility of the aldehydes shows the same trends as in Figure 11 and in the case of MHE of the aldehydes alone. The more acidic the solution is, the less volatile the aldehyde is.

4.4.3 Multiple headspace-gas extraction of aldehydes from imines at different humidity

The release of 5-methylfurfural from 6^{I}-deoxy-6^{I}-((5-methyltetrahydrofuran-2-yl)methylene)-amino-β-cyclodextrin (**13**) was investigated by MHE in solid state at different percentages of humidity. Approximately 4.10 mg of compound (**13**) was put in a small, 2 mL vial which was embedded into a 22 mL headspace vial containing 1 ml of saturated salt solution to obtain the given percentage of humidity at 60 °C and sealed using silicone septa and aluminium foil. Saturated K_2SO_4 was used to obtain 97% of humidity, saturated KNO_3 was used to obtain 47% of humidity, saturated LiCl was used to obtain 11% of humidity – all at 60 °C and no solution was used to obtain 0% of humidity in the headspace vial[77,78]. The samples were then immediately measured on SHGC using MHE using the method described in chapter 5.1 with the change that there is no equilibrium time in the beginning and the vials were then thermostated at 60 ± 0.1 °C. MHE was performed every 30 minutes during 24 hours.

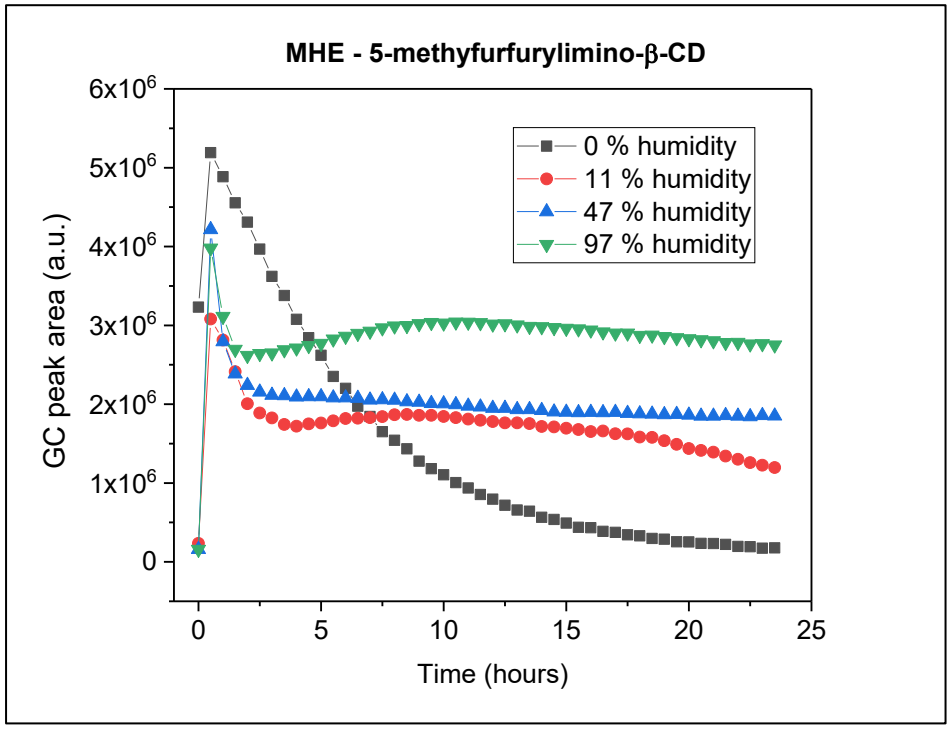


Figure 14: MHE of 6^{I}-deoxy-6^{I}-((5-methyltetrahydrofuran-2-yl)methylene)amino-β-cyclodextrin (13) at different humidity

Figure 14 shows the results of the release of 5-methylfurfural from compound (**13**) by hydrolysis of the imine bond by humidity. In the case of 0% humidity a huge peak of 5-methylfurfural release can be seen which in time drops to the zero. This jump can be explained by the fact that there is still some aldehyde left in the cyclodextrin cavity which after thermostating at 60 °C is released. In the cases where there is some humidity present, the course of the release is different. In the beginning the same jump can be seen as in the case of 0% humidity but it doesn't drop to 0 because in the meantime the imine bonds start to hydrolyze and the release of 5-methylfurfural takes place. The release process reaches a steady state in which the imine bonds are hydrolyzed and the aldehydes are slowly released from the solid powder to the headspace-gas. The percentage of humidity has an impact on the amount of the hydrolyzed imine bonds – the higher the humidity is, the more aldehyde is released from the solid state to the headspace-gas at the same time.

5 Experimental section

5.1 Instruments, general methods and chemicals

^{1}H NMR, ^{13}C NMR, 2D NMR (H,H-COSY, HSQC and HMBC) were measured on Bruker AVANCE III 600 MHz (600.17 MHz for ^{1}H, 150.04 MHz for ^{13}C) and Varian ^{UNITY}INOVA 400 (399,95 MHz for ^{1}H and 100,58 MHz for ^{13}C). For the kinetic studies the ^{1}H NMR spectra were acquired on Varian VNMRS 300 (300 MHz for ^{1}H). DMSO-d_6 and D_2O were used as the solvents. The chemical shift values (δ) are given in ppm and the values of the interaction constants (*J*) in Hz. For each substance is given its structure along with NMR numbering in chapter 5.2.2.

Headspace-gas chromatography measurements were conducted with an Agilent headspace autosampler. Sample solutions of 10 mL containing different concentrations of aroma (10–1000 ppm) were introduced into 22 mL headspace vials and sealed using silicone septa and aluminum foil. The vials were then thermostated at 30 ± 0.1 °C. After the equilibrium was established (30 min), 1 mL of vapor from the above solution was withdrawn from the vial using a gas-tight syringe and injected directly in the chromatographic column via a transfer line (250 °C). Each sample was then analyzed by gas chromatography (Perkin Elmer Autosystem XL equipped with a flame-ionization detector using a DB624 column). The GC settings were set as follows: detector temperature, 160 °C; column temperature: 160 °C for benzaldehyde, 5-methylfurfural and cinnamaldehyde and 120 °C for heptanal. The retention times under the given conditions were 2.1 min for benzaldehyde and 5-methylfurfural, 6.2 min for cinnamaldehyde and 3.1 min for heptanal.

The mass spectra were measured by the Bruker ESQUIRE 3000 ES-ion trap and the samples were ionized using an electrospray technique (ESI). The samples were dissolved in methanol.

Specific optical rotation was measured by the Rudolph Research AUTOPOL ™ III Polarimeter at 25 °C and at the wavelength of the sodium doublet. Specific optical rotation values ($[\alpha]^{25}_{D}$) are given in 10^{-1} $cm^2 \cdot g^{-1}$.

For evaporation of the solvents used a rotary vacuum evaporator from Büchi at temperatures up to 50 °C and a Glass oven B-528 Kugelrohr from Büchi at temperatures up to 110 °C.

For thin layer chromatography (TLC) DCAlufolien Keisegel 60 F265 (Merck, Darmstadt, Germany) silica gel plates were used. Carbonization in 50% sulfuric acid was used to detect the substances. As an eluent mixture for TLC propanol/water/25% aqueous ammonia/ethyl acetate 6/3/1/1 (EM1) was used.

Anhydrous DMF was prepared by distillation with P_2O_5 at reduced pressure and was stored over molecular sieves 3 Å under argon atmosphere. Organic solvents were distilled before use. β-CD was purchased from WAKO Chemicals (Germany). Other reagents were purchased from common commercial sources and used without further purification (Sigma-Aldrich, Penta).

Aqueous 0.1M phosphate buffers were prepared by mixing 0.1M solutions of H_3PO_4, KH_2PO_4, K_2HPO_4 and K_3PO_4 according to the ratios shown in Table 8. The exact pH value was tuned with titration with the help of pH meter. Note that all the buffers can be prepared by mixing those phosphate solutions in different ratio and tuning with stronger or weaker bases or acids.

Table 8: Preparation of 0.1M aqueous phosphate buffers

pH	H_3PO_4	KH_2PO_4	K_2HPO_4	K_3PO_4
1.08	1	—	—	—
2.00	1	—	1	—
3.00	dropwise	1	—	—
4.00	—	1	dropwise	—
5.00	—	1	dropwise	—
6.00	—	10	1	—
7.00	—	15	5	3
8.00	—	3	17	dropwise
9.00	—	dropwise	1	—
10.00	—	—	1	dropwise
11.00	—	10	3	10
12.00*	—	—	dropwise	1
12.80	—	—	—	1

5.2 Synthesis of compounds

5.2.1 Synthesis of cyclodextrin precursors

6^I-*O*-*p*-Toluenesulfonyl-β-cyclodextrin (1)

Compound **1** was prepared according to the article[42], which was modified. A suspension of β-CD (90.80 g, 72 mmol) and *p*-toluenesulfonylchloride (18.20 g, 93.6 mmol) in 1.70 L of water was stirred for 2 hours at laboratory temperature. A solution of NaOH (35.4 g, 885.6 mmol) in 300 mL of water was added to the suspension, after 10 minutes the unreacted *p*-toluenesulfonylchloride was filtrated and the filtrate was neutralized with 10M HCl. The excluded precipitate was left in the refrigerator overnight, the next day it was filtrated and dried. The reaction was monitored by TLC

with EM1 and detected with 50% H_2SO_4. The crude product was refluxed in 50% aqueous MeOH (the volume is ten times the weight of the crude product), and crystallized for 1 hour at laboratory temperature. The thrice repeated crystallization gave 21.91 g of white crystalline **1** with the yield of 23.6%. NMR spectra are in agreement with literature[42].

6^I-Azido-6^I-deoxy-β-cyclodextrin (2)

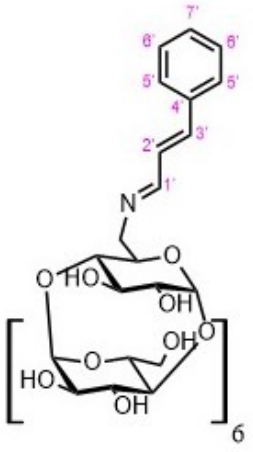
Compound **2** was prepared according to the procedure published in the article[43]. Compound **1** (10.36 g, 8,02 mmol) and NaN_3 (10.53g, 160,39 mmol) was dissolved in 100 mL of DMF, was stirred at 80°C overnight and the next day the solvent was removed by evaporation from the reaction mixture. The reaction was monitored by TLC with EM1 and detected with 50% H_2SO_4. The procedure was changed by modification of the purification of the crude product, which was purified on column chromatography with reversed phase silica gel obtaining 9.14 g of **2** with the yield of 98.2%. NMR spectra are in agreement with literature[43].

6^I-Amino-6^I-deoxy-β-cyclodextrin (3)

Compound **3** was synthesized according to the literature[44]. Compound **2** (3.48 g, 3,00 mmol) was dissolved in 70 mL of DMF, triphenylphosphine (1.73 g, 7,6 mmol) was added and the reaction mixture was stirred at 90 °C for 22,5 hours. The reaction was monitored by TLC with EM1 and detected with 50% H_2SO_4. After full conversion the reaction was poured into acetone (1.25 L), the excluded precipitate was filtrated and washed 2 times with acetone (100 mL). The product was purified on cation exchanger with 5% aqueous ammonia. The lyophilisation of the acquired solution gave 3.08 g of white amorphous **3** with the yield of 90.4%. NMR spectra are in agreement with literature[44].

5.2.2 Synthesis of imine derivatives of β-cyclodextrin

6^I-Deoxy-6^I-(3-penylallylidene)amino-β-cyclodextrin (4)



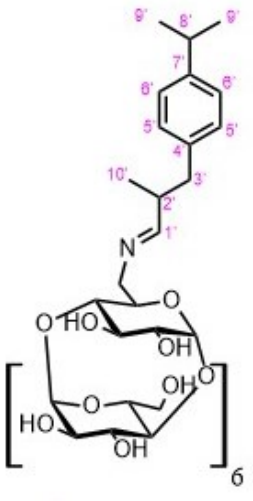
Compound **3** (210.9 mg, 0.186 mmol) and *trans*-cinnamaldehyde (0.7 mL, 5.58 mmol) was refluxed in 100 mL of MeOH under argon overnight. The reaction was monitored by MS and after full conversion to imine the solvent was removed by evaporation. The remaining aldehyde was extracted ten times with hexane (10 mL) and product was dried on Kugelrohr at 110 °C. The reaction gave 222.4 mg of yellowish powder **4** with the yield of 96%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.44 (s, 1H, **H1'**), 8.07 (d, J = 8.6 Hz, 1H, **H7'**), 7.60 (d, J = 7.4 Hz, 2H, **H5'**), 7.52 (d, J = 7.4 Hz, 2H, **C6'**), 6.95 -6.91 (m, 1H, **H3'**), 6.41 (d, J = 18 Hz, 1H, **H2'**), 6.05 – 5.92 (m, 14H, **2,3-OH**), 4.90 – 4.81 (m, 7H, **H1**), 4.52 – 4.42 (m, 6H, **6-OH**), 3.88 – 3.30 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.50 (**C1'**), 137.34 (**C4'**), 129.31 (**C2'**), 129.13 (**C3'**), 128.71 (**C7'**), 127.69 (**C6'**), 127.58 (**C5'**), 102.56 – 102.25 (**C1^{I}**), 83.85 – 82.01 (**C4^{I}**), 73.64 – 70.95 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 60.96 – 60.40 (**C6^{I}**)

For $C_{51}H_{77}NO_{34}$ calculated M_r: 1248.15, ESI-MS: *m/z* 1270 $[M + Na]^+$, $[\alpha]^{25}_D$ + 103.4°

6^{I}-Deoxy-6^{I}-((3-(4-isopropylphenyl)-2-methylpropylidene)amino)-β-cyclodextrin (5)



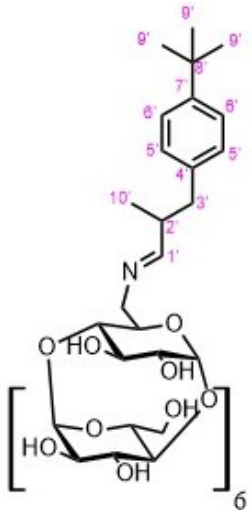
From compound **3** (211.0 mg, 0.186 mmol) and 3-(4-isopropylphenyl)-2-methylpropanal (cyclamen aldehyde) (1.18 mL, 5.58 mmol) according to the synthesis route of **4**; 200.7 mg of **5** was afforded as a white powder with the yield of 83%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.22 (s, 1H, **H1'**), 7.86 (d, *J* = 8.5 Hz, 2H, **H6'**), 7.67 (d, *J* = 8.4 Hz, 2H, **H5'**), 5.81 (bs, 14H, **2,3-OH**), 4.92 – 4.74 (m, 7H, **H1**), 4.62 – 4.24 (m, 6H, **6-OH**), 3.89 – 3.08 (m, 44H, **H2**, **H3**, **H4**, **H5**, **H6**, **H2'**, **H8'**), 2.73 – 2.70 (m, 2H, **H3'**), 1.38 (s, 6H, **H9'**), 0.93 (d, *J* = 6.6 Hz, 3H, **H10'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.28 (**C1'**), 132.79 (**C5'**), 130.53 (**C6'**), 103.14 – 102.51 (**C1^{I}**), 84.58 – 82.27 (**C4^{I}**), 74.27 – 71.56 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 61.38 – 60.59 (**C6^{I}**), 56.68 (**C2'**), 56.21 (**C3'**), 34.94 (**C8'**), 27.39 (**C9'**), (**C10'**)

For $C_{55}H_{87}NO_{34}$ calculated M_r: 1306.27, ESI-MS: *m/z* 1328 $[M + Na]^+$, $[\alpha]^{25}_D$ + 111.7°

6^{I}-((3-(4-(*tert*-Butyl)phenyl)-2-methylpropylidene)amino)-6^{I}-deoxy-β-cyclodextrin (6)



From compound **3** (211.0 mg, 0.186 mmol) and 3-(4-(*tert*-butyl)phenyl)-2-methylpropanal (lilial) (0.84 mL, 5.58 mmol) according to the synthesis route of **4**; 223.6 mg of **5** was afforded as a powder with the yield of 91%.

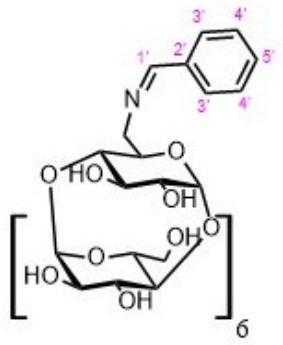
^{1}H NMR (600 MHz, DMSO-d_6): δ 8.47 (s, 1H, **H1'**), 7.22 (d, *J* = 8.1 Hz, 2H, **H6'**), 7.07 (d, *J* = 7.9 Hz, 2H, **H5'**), 5.81 (bs, 14H, **2,3-OH**), 4.85 – 4.64 (m, 7H, **H1**), 4.31 – 4.02 (m, 6H, **6-OH**), 3.77 – 3.03 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 2.59 – 2.55 (m, 2H, **H3'**), 1.24 (s, 9H, **H9'**), 0.88 (d, *J* = 6.8 Hz, 3H, **H10'**), (1H, **H2'** peak is under DMSO peak at 2.54)

^{13}C NMR (125 MHz, DMSO-d_6): δ 167.10 (**C1'**), 162.06 (**C7'**), 139.74 (**C7'**), 137.27 (**C4'**), 129.19 (**C5'**), 125.26 (**C6'**), 129.99 (**C4'**), 103.05 – 102.45 (**C1^{I}**), 85.25 – 81.90

(**C4^{I}**), 75.86 – 81.90 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 61.13 – 60.04 (**C6^{I}**), 41.86 (**C2'**), 39.27 (**C3'**), 34.94 (**C8'**), 32.25 (**C9'**), 31.88 (**C8'**)14.94 (**C10'**)

For $C_{56}H_{89}NO_{34}$ calculated M_r: 1320.30, ESI-MS: *m/z* 1342 $[M + Na]^+$, $[\alpha]^{25}_D$ + 103.9°

6^{I}-Benzylideneamino-6^{I}-Deoxy-β-cyclodextrin (7)



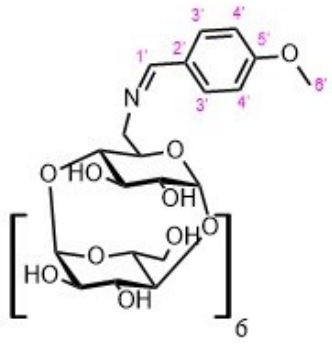
From compound **3** (155.3 mg, 0.137 mmol) and benzaldehyde (0.28 mL, 2.74 mmol) according to the synthesis route of **4**; 144.3 mg of **7** was afforded as a white powder with the yield of 86%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.33 (s, 1H, **H1'**), 7.76 – 7.75 (m, 2H, **H3'**), 7.45 – 7.44 (m, 3H, **H4'**, **H5'**), 5.91 – 5.75 (m, 14H, **2,3-OH**), 4.96 – 4.80 (m, 7H, **H1**), 4.51 – 4.33 (m, 6H, **6-OH**), 3.82 – 3.32 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.49 (**C1'**), 130.92 (**C3'**), 128.93 (**C4'**), 128.42 (**C5'**), 102.60 – 101.98 (**C1^{I}**), 83.99 – 81.71 (**C4^{I}**), 73.64 – 72.42 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 59.97 – 60.84 (**C6^{I}**)

For $C_{49}H_{75}NO_{34}$ calculated M_r: 1222.11, ESI-MS: *m/z* 1244 $[M + Na]^+$, $[\alpha]^{25}_D$ + 103.4°

6^{I}-Deoxy-6^{I}-(4-methoxybenzylidene)amino-β-cyclodextrin (8)



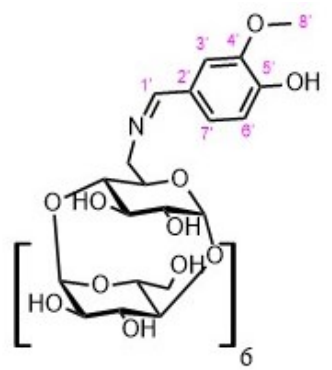
From compound **3** (211 mg, 0.186 mmol) and 4-methoxybenzaldehyde (4-anisaldehyde) (0.35 mL, 2.80 mmol) according to the synthesis route of **4**; 191.1 mg of **8** was afforded as a white powder with the yield of 82%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.22 (s, 1H, **H1'**), 7.86 (d, *J* = 8.5 Hz, 2H, **H3'**), 7.11 (d, *J* = 8.4 Hz, 2H, **H4'**), 5.81 (bs, 14H, **2,3-OH**), 4.92 – 4.74 (m, 7H, **H1**), 4.64 – 4.24 (m, 6H, **6-OH**), 3.89 (s, 3H, **H6'**), 3.78 – 3.13 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 161.75 (**C1'**), 161.49 (**C5'**), 132.27 (**C3'**), 129.46 (**C2'**), 114.96 (**C4'**), 102.62 – 102.03 (**C1^{I}**), 84.06 – 81.74 (**C4^{I}**), 73.73 – 71.05 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 60.85 – 59.98 (**C6^{I}**), 55.69 (**C6'**)

For $C_{50}H_{77}NO_{35}$ calculated M_r: 1252.14, ESI-MS: *m/z* 1274 $[M + Na]^+$, $[\alpha]^{25}_D$ + 102.4°

6^{I}-Deoxy-6^{I}-(4-hydroxy-3-methoxybenzylidene)amino-β-cyclodextrin (9)



Compound **3** (211 mg, 0.186 mmol) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) (596 mg, 3.72 mmol) was refluxed in 100 mL of MeOH under argon overnight. The remaining aldehyde was extracted ten times with toluene (10 mL) and product was dried on

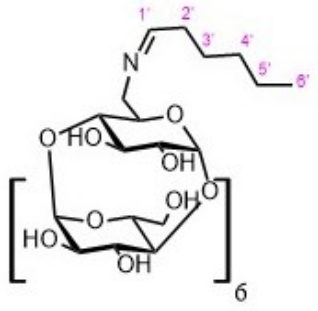
Kugelrohr at 110 °C. The reaction gave 227.1 mg of yellow powder **9** with the yield of 96%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.17 (s, 1H, **H1'**), 7.37 (s, 1H, **H3'**), 7.17 (dd, J = 8.3, 2.1 Hz, 1H, **H6'**), 7.05 (d, J = 2.1 Hz, 1H, **H7'**), 5.19 (bs, 14H, **2,3-OH**), 4.94 – 4.84 (m, 7H, **H1**), 4.72 – 4.24 (m, 6H, **6-OH**), 3.96 – 3.32 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 3.82 (s, 3H, **H8'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.27 (**C1'**), 151.37 (**C5'**), 148.47 (**C4'**), 130.30 (**C2'**), 123.45 (**C7'**), 117.33 (**C6'**), 110.37 (**C3'**), 102.66 – 102.13 (**C1^{I}**), 84.36 – 81.89 (**C4^{I}**), 73.70 – 71.09 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 61.14 – 60.13 (**C6^{I}**), 55.20 (**C8'**)

For $C_{50}H_{77}NO_{36}$ calculated M_r: 1268.14, ESI-MS: m/z 1290 $[M + Na]^+$, $[\alpha]^{25}_D$ +88.6°

6^{I}-Deoxy-6^{I}-hexylideneamino-β-cyclodextrin (10)



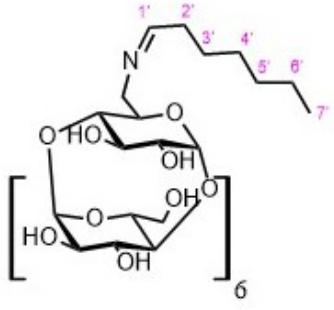
From compound **3** (211 mg, 0.186 mmol) and hexanal (0.46 mL, 3.72 mmol) according to the synthesis route of **4**; 201.3 mg of **5** was afforded as a white crystalline with the yield of 89%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 6.59 (s, 1H, **H1'**), 6.35 – 5.20 (m, 14H, **2,3-OH**), 5.05 – 4.86 (m, 7H, **H1**), 4.54 – 4.03 (m, 6H, **6-OH**), 3.82 – 2.95 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 2.03 – 1.97 (m, 2H, **H2'**), 1.42 – 1.21 (m, 6H, **H3'**, **H4'**, **H5'**), 0.83 (s, 3H, **H6'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 156.21 (**C1'**), 102.83 – 102.18 (**C1^{I}**), 82.35 – 81.51 (**C4^{I}**), 73.53 – 72.26 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 60.80 – 69.91 (**C6^{I}**), 36.87 (**C2'**), 31.70 (**C3'**), 25.71 (**C4'**), 22.43 (**C5'**), 14.38 (**C6'**)

For $C_{48}H_{81}NO_{34}$ calculated M_r: 1216.15, ESI-MS: *m/z* 1238 $[M + Na]^+$, $[\alpha]^{25}_D$ + 105.1°

6^{I}-Deoxy-6^{I}-heptylideneamino-β-cyclodextrin (11)



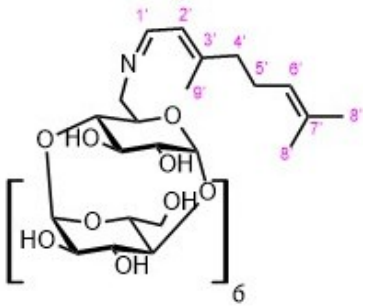
From compound **3** (211 mg, 0.186 mmol) and heptanal (0.53 mL, 3.72 mmol) according to the synthesis route of **4**; 221.1 mg of **11** was afforded as a white powder with the yield of 97%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 7.81 (d, J – 27.2 Hz, 1H, **H1'**), 6.33 – 5.72 (m, 14H, **2,3-OH**), 4.99 – 4.79 (m, 7H, **H1**), 4.49 (bs, 6H, **6-OH**), 3.59 – 3.27 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 2.24 – 2.17 (m, 2H, **H3'**), 2.00 – 1.89 (m, 2H, **H2'**), 1.44 – 1.08 (m, 6H, **H4'**, **H5'**, **H6'**), 0.88 (s, 3H, **H7'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 165.83 (**C1'**), 102.92 – 102.19 (**C1^{I}**), 82.40 – 81.67 (**C4^{I}**), 73.51 – 72.45 (**C2^{I}**, **C3^{I}**, **C5^{I}**), 60.72 – 59.99 (**C6^{I}**), 38.01 (**C2'**), 31.60 (**C3'**), 29.24 (**C4'**), 26.42 (**C5'**), 22.49 (**C6'**), 14.39 (**C7'**)

For $C_{49}H_{83}NO_{34}$ calculated M_r: 1230.18, ESI-MS: *m/z* 1252 $[M + Na]^+$, $[\alpha]^{25}_D$ + 103.9°

6^I-Deoxy-6^I-(3,7-dimethylocta-2,6-dien-1-ylidene)amino-β-cyclodextrin (12)



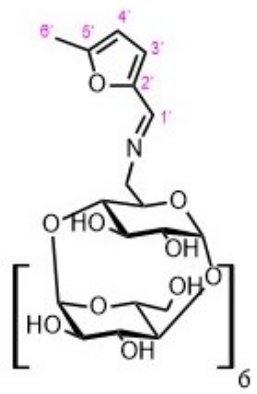
From compound **3** (211 mg, 0.186 mmol) and 3,7-dimethylocta-2,6-dienal (citral) (1.00 mL, 5.58 mmol) according to the synthesis route of **4**; 188.9 mg of **12** was afforded as a yellowish powder with the yield of 80%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.48 (s, 1H, **H1'**), 6.16 – 5.83 (m, 14H, **2,3-OH**), 5.89 (bs, 1H, **H2'**), 5.17 – 5.09 (m, 1H, **H6'**) 4.86 – 4.69 (m, 7H, **H1**), 4.60 – 4.22 (m, 6H, **6-OH**), 3.83 – 3.09 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 2.19 – 2.09 (m, 4H, **H4'**, **H5'**), 1.69 (s, 3H, **H9'**), 1.62 (s, 6H, **H8'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.80 (**C1'**), 126.26 – 123.64 (**C2'**, **C3'**, **C6'**, **C7'**), 102.59 – 102.09 (**C1^I**), 84.18 – 81.82 (**C4^I**), 73.91 – 71,04 (**C2^I**, **C3^I**, **C5^I**), 60.87 – 60.34 (**C6^I**), 26.78 – 26.16 (**C4'**, **C5'**), 25.97 (**C9'**), 18.05 (**C8'**)

For $C_{52}H_{85}NO_{34}$ calculated M_r: 1268.23, ESI-MS: *m/z* 1290 $[M + Na]^+$, $[\alpha]^{25}_D$ + 94.6°

6^I-Deoxy-6^I-((5-methyltetrahydrofuran-2-yl)methylene)amino-β-cyclodextrin (13)



From compound **3** (211 mg, 0.186 mmol) and 5-methyltetrahydrofuran-2-carbaldehyde (5-methylfurfural) (0.56 mL, 5.58 mmol) according to the synthesis route of **4**; 191.2 mg of **13** was afforded as a brownish powder with the yield of 83%.

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.03 (s, 1H, **H1'**), 6.79 (d, *J* = 3.3 Hz, 1H, **H3'**), 6.22 (d, *J* = 3.1 Hz, 1H, **H4'**), 5.81 (bs, 14H, **2,3-OH**), 4.88 – 4.79 (m, 7H, **H1**), 4.43 (bs, 6H, **6-OH**), 3.88 – 3.28 (m, 42H, **H2**, **H3**, **H4**, **H5**, **H6**), 2.32 (s, 3H, **H6'**)

^{13}C NMR (125 MHz, DMSO-d_6): δ 162.58 (**C1'**), 151.45 (**C5'**), 115.99 (**C2'**), 111.24 (**C3'**), 108.66 (**C4'**), 102.64 – 102.19 (**C1^I**), 83.94 – 81.92 (**C4^I**), 73.73 – 72.47 (**C2^I**, **C3^I**, **C5^I**), 60.91 – 60.84 (**C6^I**), 13.93 (**C6'**)

For $C_{48}H_{75}NO_{35}$ calculated M_r: 1226.10, ESI-MS: *m/z* 1248$[M + Na]^+$, $[\alpha]^{25}_D$ + 97.2°

6 Conclusion

In the synthetic part of the thesis, a complete series of ten monosubstituted imino derivatives of β-cyclodextrin was successfully prepared. The individual synthesis steps included monotosylation of β-CD in position 6, substitution of the *p*-toluenesulfonate group with an azide group and reduction of the azide to amine. Subsequently Schiff bases of aldehydes and 6^{I}-amino-6^{I}-deoxy-β-cyclodextrin were prepared as pro-fragrances.

The release study of the aldehydes from the prepared compounds was studied by ^{1}H NMR spectroscopy in 0.1M aqueous phosphate buffers at different pH values and the half-time of the hydrolysis was calculated for ten pH values. The studies showed that the more acidic the buffer is, the faster the hydrolysis is. For pH below 4 the half-life was not measurable with the used ^{1}H NMR spectroscopy technique because of the fast hydrolysis. In basic conditions the hydrolysis was slow and the half-life values are in hours and days.

The fragrance release was also studied by SHGC in buffers at different pH values and different humidity percentages. It was observed that the volatility of the aldehyde depends on the pH of the solution. In acidic solutions, the aldehyde is less volatile than in basic solutions because of the higher amount of hydrogen bonds. The humidity studies showed that the higher the humidity percentage is, the faster the hydrolysis is. Less humidity percentage resulted in less aldehyde amount in the headspace area.

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